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(54) Title: AGONIST PEPTIDE DIMERS

(57) Abstract

The present invention is directed to the dimerization of agonists and antagonists of cell surface receptors and particularly to peptide dimers which behave as cell surface receptor agonists in their dimeric form. Such receptors belong to the dimerization-mediated activation class often observed among receptors for growth and differentiation factors. The agonists of this class of receptors is understood to effect dimerization of the receptor and thus signal initiation. The present invention exemplifies dimers of erythropoietin (EPO) agonists and antagonists comprising a core amino acid sequence of X₃X₄X₅GPX₆TWX₇X₈ (SEQ ID NO: 1) wherein each amino acid is indicated by standard one letter abbreviation; X₃ can be C, A, α -amino- γ -bromobutyric acid or Hoc; X₄ can be R, H, L or W; X₅ can be M, F, or I; X₆ is independently selected from any one of the 20 genetically coded L-amino acids or the stereoisomeric D-amino acids; X₇ can be D, E, I, L or V; and X₈ can be C, A, α -amino- γ -bromobutyric acid or Hoc, provided that either X₃ or X₈ is C or Hoc.

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AGONIST PEPTIDE DIMERSFIELD OF THE INVENTION

5 The present invention is directed to the dimerization of agonists and antagonists of cell surface receptors and particularly to peptide dimers which behave as cell surface receptor agonists in their dimeric form. Such receptors belong to the 10 dimerization-mediated activation class often observed among receptors for growth and differentiation factors. The agonists of this class of receptors is understood to effect dimerization of the receptor and thus signal 15 initiation. The present invention exemplifies dimers of erythropoietin (EPO) agonists and antagonists comprising a core amino acid sequence of $X_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 1) wherein each amino acid is indicated by standard one letter abbreviation; X_3 can be C, A, α -amino- γ -bromobutyric acid or Hoc; X_4 can be R, H, L or W; X_5 can 20 be M, F, or I; X_6 is independently selected from any one of the 20 genetically coded L-amino acids or the stereoisomeric D-amino acids; X_7 can be D, E, I, L or V; and X_8 can be C, A, α -amino- γ -bromobutyric acid or Hoc, provided that either X_3 or X_8 is C or Hoc.

25

BACKGROUND OF THE INVENTION

30 Erythropoietin (EPO) is a glycoprotein hormone with an approximate molecular weight of 34,000 daltons. The primary role of EPO, which is synthesized in the kidneys of mammals, is to stimulate mitotic cell division and differentiation of erythrocyte precursor cells. As a result, EPO acts to stimulate and to

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1 regulate the production of erythrocytes. Erythrocytes,
and the hemoglobin contained therein, play a central
role in supplying oxygen to the body. Thus, the
5 stimulation of erythrocyte production is able to
increase the oxygen-carrying capacity of the blood.

10 During normal conditions, EPO is present in
very low concentrations in plasma. Under hypoxic
conditions, the amount of EPO in the circulation is
increased in response to reduced O₂ blood levels.
Hypoxia may be caused from various conditions including
15 the loss of large amounts of blood, destruction of red
blood cells by over-exposure to radiation or
chemotherapeutic agents, reduction in oxygen intake due
to high altitudes or prolonged unconsciousness, or by
various forms of anemia. As the hypoxic condition
diminishes, the amount of EPO produced subsequently
diminishes.

20 Because of the essential role of EPO in red
blood cell formation, the hormone is useful in both the
diagnosis and the treatment of blood disorders
characterized by low or defective red blood cell
production. Recent studies provide a basis for the
efficacy of EPO therapy in a variety of disease states,
25 disorders, and states of hematologic irregularity,
including: beta-thalassemia (See, Vedovato et al.
(1984) Acta. Haematol. 71:211-213); cystic fibrosis
(See, Vichinsky et al. (1984) J. Pediatric 105:15-21);
pregnancy and menstrual disorders (See, Cotes et al.
30 (1983) Brit. J. Obstet. Gynaecol. 90:304-311); early
anemia of prematurity (See, Haga et al. (1983) Acta
Pediatri. Scand. 72:827-831); spinal cord injury (See,

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1 Claus-Walker et al. (1984) Arch. Phys. Med. Rehabil.
65:370-374); space flight (See, Dunn et al. (1984) Eur.
J. Appl. Physiol. 52:178-182); acute blood loss (See,
5 Miller et al. (1982) Brit. J. Haematol. 52:545-590);
aging (See, Udupa et al. (1984) J. Lab. Clin. Med.
10 103:574-588); various neoplastic disease states
accompanied by abnormal erythropoiesis (See, Dainiak et
al. (1983) Cancer 5:1101-1106); and renal insufficiency
(See, Eschbach et al. (1987) N. Eng. J. Med. 316:73-78).

10 Although purified, homogenous EPO has been
characterized, little is known about the mechanism of
EPO-induced erythroblast proliferation and
differentiation. The specific interaction of EPO with
15 progenitor cells of immature red blood cells, platelets,
and megakaryocytes has not been described. This is due
in part, to the small number of surface EPO receptor
molecules on normal erythroblasts and on the
erythroleukemia cell lines. See Krantz and Goldwasser
20 (1984) Proc. Natl. Acad. Sci. USA, 81:7574-7578; Branch
et al. (1987) Blood 69:1782-1785; Mayeux et al. (1987)
25 FEBS Letters 211:229-233; Mufson and Gesner (1987) Blood
69:1485-1490; Sakaguchi et al. (1987) Biochem. Biophys.
Res. Commun. 146:7-12; Sawyer et al. (1987) Proc. Natl.
Acad. Sci. USA 84:3690-3694; Sawyer et al. (1987) J.
30 Biol. Chem. 262:5554-5562; and Todokoro et al. (1988)
Proc. Natl. Acad. Sci. USA 84:4126-4130. The DNA
sequences and encoded peptide sequences for murine and
human EPO receptor proteins have been described. See,
D'Andrea et al. PCT Patent Publication No. WO 90/08822
(published 1990).

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1 The EPO-receptor (EPO-R) belongs to the class
of growth-factor-type receptors which are activated by a
ligand-induced protein dimerization. Other hormones and
5 cytokines such as human growth hormone (hGH),
granulocyte colony stimulating factor (G-CSF), epidermal
growth factor (EGF) and insulin can cross-link two
receptors resulting in juxtaposition of two cytoplasmic
10 tails. Many of these dimerization-activated receptors
have protein kinase domains within the cytoplasmic tails
that phosphorylate the neighboring tail upon
dimerization. While some cytoplasmic tails lack
intrinsic kinase activity, these function by association
with protein kinases. The EPO receptor is of the latter
15 type. In each case, phosphorylation results in the
activation of a signaling pathway.

20 In accordance with the present invention, it
has been discovered that the dimerization of peptide
agonists and antagonists of dimerization-mediated
receptors, such as EPO-R, increase the biological
efficacy relative to the biological activity of the
25 'monomeric' agonists and alters the properties of the
antagonists such that, these dimers function as
agonists, exhibiting biological activity.

30 SUMMARY OF THE INVENTION

35 In a first embodiment, the present invention
is directed to peptide dimers which behave as cell-
surface receptor agonists, dimers which exhibit binding
and signal initiation of growth factor-type receptors.
In one embodiment, the present invention provides
peptide dimers which behave as EPO agonists. These

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1 dimers have two 'monomeric' peptide units of 10 to 40 or
more amino acids, preferably 14 to about 20 residues in
length, comprising a core amino acid sequence of
5 $X_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 1) where each amino acid is
indicated by standard one letter abbreviation; X_3 can be
C, A, α -amino- γ -bromobutyric acid, or Hoc, where Hoc is
homocysteine; X_4 can be R, H, L, or W; X_5 can be M, F,
or I; X_6 is independently selected from any one of the
10 20 genetically coded L-amino acids or the stereoisomeric
D-amino acids; X_7 can be D, E, I, L, or V; and X_8 can be
C, A, α -amino- γ -bromobutyric acid, or Hoc, where Hoc is
homocysteine, provided that either X_3 or X_8 is C or Hoc.
Preferably, the monomeric peptide unit of the dimer
15 comprises a core sequence $YX_2X_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID
NO: 2) where each amino acid is indicated by standard
one letter abbreviation; each X_2 and X_8 is independently
selected from any one of the 20 genetically coded L-
amino acids; X_3 can be C, A, α -amino- γ -bromobutyric
20 acid, or Hoc, where Hoc is homocysteine; X_4 can be R, H,
L, or W; X_5 can be M, F, or I; X_7 can be D, E, I, L, or
V; and X_8 can be C, A, α -amino- γ -bromobutyric acid, or
Hoc, where Hoc is homocysteine, provided that either X_3
or X_8 is C or Hoc.

25 More preferably, the monomeric peptide unit of
the dimer comprises a core sequence of amino acids
 $X_1YX_2X_3X_4X_5GPX_6TWX_7X_8X_9X_{10}X_{11}$ (SEQ ID NO: 3), where each
amino acid is indicated by standard one letter
abbreviation; each X_1 , X_2 , X_5 , X_6 , X_9 , X_{10} , and X_{11} is
30 independently selected from any one of the 20
genetically coded L-amino acids; X_3 can be C, A, α -
amino- γ -bromobutyric acid, or Hoc, where Hoc is

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1 homocysteine; X_4 can be R, H, L, or W; X_5 can be M, F, or I; X_7 can be D, E, I, L or V; and X_9 can be C, A, α -amino- γ -bromobutyric acid, or Hoc, where Hoc is homocysteine, provided that either X_3 or X_9 is C or Hoc.

5 In a more preferred embodiment, both X_3 and X_9 are C and thus, the monomeric peptide unit of the dimer comprises a core sequence of amino acids

10 $X_1YX_2CX_4X_5GPX_6TWX_7CX_9X_{10}X_{11}$ (SEQ ID NO: 4). More preferably, the monomeric peptide unit comprises a core sequence of amino acids $X_1YX_2CX_4X_5GPX_6TWX_7CX_9X_{10}X_{11}$ (SEQ ID NO: 5), where X_4 can be R or H; X_5 can be F or M; X_6 can be I, L, T, M, or V; X_7 is D or V; X_9 can be G, K, L, Q, R, S, or T; and X_{10} can be A, G, P, R, or Y. In a most preferred embodiment, the monomeric peptide unit of the dimer will comprise a core sequence of amino acids $X_1YX_2CX_4X_5GPX_6TWX_7CX_9X_{10}X_{11}$ (SEQ ID NO: 6), where X_1 can be D, E, L, N, S, T, or V; X_2 can be A, H, K, L, M, S, or T; X_4 is R or H; X_5 can be K, R, S, or T; and X_{10} is P. Particularly preferred monomeric peptide units of the dimers include:

20

GGLYLCRFGPVTWDCGYKGG	(SEQ ID NO: 7);
GGTYSCHFGPLTWVCKPQGG	(SEQ ID NO: 8);
GGDYHCRMGPLTWVCKPLGG	(SEQ ID NO: 9);
VGNYMCHFGPITWVCRPGGG	(SEQ ID NO: 10);
GGVYACRMGPITWVCSPLGG	(SEQ ID NO: 11);
VGNYMAHMGPIIWVCRPGG	(SEQ ID NO: 12);
GGTYSCHFGPLTWVCKPQ	(SEQ ID NO: 13);
GGLYACHMGPMTWVCQPLRG	(SEQ ID NO: 14);
TIAQYICYMGPETWECRSPKA	(SEQ ID NO: 15);
YSCHFGPLTWVCK	(SEQ ID NO: 15);
YCHFGPLTWVC	(SEQ ID NO: 17); and

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SCHFGPLTWVCK

(SEQ ID NO: 18).

Other particularly preferred monomeric peptide units of the present dimers include peptides comprising a core sequence of the formula $(AX_2)_nX_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 19) wherein X_2 through X_8 are as previously defined herein (SEQ ID NO: 2), n is 1 or 0 and A is any one of the naturally occurring L-amino acids except Y (tyrosine); n is defined herein as the number of occurrences of (AX_2) which can be 1 or none in the core sequence. When (AX_2) is present, i.e. when $n = 1$, A is not tyrosine and A is not any non-naturally occurring aromatic amino acid analog. Such monomeric peptide units of the dimers of this invention can be prepared by truncating the peptides of Fig. 9, for example, from the N-terminus to delete the Y, tyrosine residue in SEQ ID NOS. 21 - 93. Such monomeric peptides can also be prepared by substitution of Y in position A in the peptides of Fig. 9.

20

In accordance with the present invention the monomeric units of the dimers can be the same or different.

25

In a preferred embodiment polyethylene glycol (PEG) is employed as a linker to form the dimeric peptides of the present invention through a covalent bond.

30

In another embodiment, the present invention is directed to pharmaceutical compositions comprising at least one dimer peptide of the invention and a pharmaceutical carrier.

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1 In a further embodiment, the present invention provides a method for therapeutically treating a mammal having a condition resulting from a hormone or growth factor deficiency by administration of at least one of 5 the dimer peptides of the present invention.

10 In a still further embodiment, a method for therapeutically treating a mammal having a condition resulting from a deficiency of EPO or from reduced levels of blood oxygen caused by a decrease in 15 erythrocyte number is provided.

20 In another embodiment of this invention, a method is provided for preparing agonists of cell-surface receptors wherein agonists of the class of cell-surface or dimerization-mediated receptors are dimerized to enhance the in vitro or in vivo biological activity 25 of the cell-surface receptor relative to the monomeric agonists from which the dimer is derived. This method is also directed to the preparation of agonists of such growth-factor-type receptors by dimerizing antagonists of these receptors; the dimerized 'antagonists' thereby exhibit agonist biological activity in vitro and in vivo. In a preferred embodiment, the present method is directed to the preparation of EPO-R dimer agonists from monomeric EPO-R antagonists.

BRIEF DESCRIPTION OF THE DRAWINGS

30 Fig. 1 shows a major peak, with a retention time of 37 minutes, of the dimerized EPO peptide, GGTYSCHFGPLTWVCKPQGG (SEQ ID NO: 8)

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Fig. 2 shows a major peak, with a retention time of 48 minutes, following purification of the dimerized EPO peptide, (SEQ ID NO: 8).

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Fig. 3 depicts the MALDI-TOF mass spectral analysis of the dimerized peptides, including peptide (SEQ ID NO: 8), GGTYSCHFGPLTWVCKPQ (SEQ ID NO: 13) and SCHFGPLTWVCK (SEQ ID NO: 18).

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Fig. 4 shows the SDS-PAGE analysis of DPDDB crosslinking of EPO binding protein (EBP) in the presence and absence of EPO agonist peptides.

15

Fig. 5 demonstrates equilibrium EPO binding to immobilized EPO binding protein. Panel A represents the equilibrium binding data and Panel B (inset) is the linear transformation (Scatchard) of the data set in Panel A.

20

Fig. 6 depicts the results of a competitive binding assay run on the EPO agonist peptide (SEQ ID NO: 8) in competitive binding with [¹²⁵I]EPO to EBP beads (Panel A); and EPO responsive cell proliferation studies in FDC-P1 derived cell lines containing either a human (Panel B) or murine EPO receptor (Panel C).

25

Fig. 7 is a graphic representation of the results of the exhypoxic mouse bioassay; stimulation of the incorporation of [⁵⁵Fe] into nascent red blood cells by EPO, peptide (SEQ ID NO: 8) (Panel A) and peptide (SEQ ID NO: 8) dimer (Panel B).

30

Fig. 8 demonstrates the effect of PEG dimerization of peptide (SEQ ID NO: 18) activity in EPO responsive cell proliferation studies in FDC-P1 derived cell lines containing a human EPO receptor.

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Fig. 9 provides the sequences of representative monomeric peptides of the present invention.

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DETAILED DESCRIPTION OF THE INVENTION

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The present invention is directed to peptide dimers which behave as cell surface receptor agonists, dimers which exhibit binding and signal initiation of growth-factor-type receptors. Sometimes called cell-surface receptors, growth-factor-type receptors or dimerization-mediated activator-receptors, these are a class of molecules which are understood to be activated by ligand-induced or ligand stabilized dimerization.

15

Agonists of such receptors typically include large polypeptide hormones including the cytokines, insulin and various other growth or differentiation factors. The agonists are understood to induce dimerization of the receptor and thereby effect signal initiation. Such agonists are believed to effectively cross-link two receptors resulting in the repositioning of cytoplasmic tails which may directly or indirectly effect phosphorylation of the cytoplasmic tails and activation of a signaling pathway.

20

The present invention specifically includes those molecules which behave as agonists of cell-surface receptors when dimerized in accordance with this invention. Such dimer agonists can include 'monomeric' units which exhibit agonist or antagonist activity for the related receptor molecule and may be the same or different. The dimers are preferably peptides but may alternatively be small molecule pharmacophores. These molecules when dimerized exhibit agonist activity of

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1 cell-surface receptors in vitro and in vivo. Such
receptors include, for example, EPO, GM-CSF, G-CSF, M-
CSF, GH, EGF, PDGF, VEGF, Insulin and FGF. Other
5 receptors which are activated by heterodimerization or
multimerization may also be subject to activation by
this mechanism including, IL-3, IL-5, IL-6, IL-2 and
TPO. The dimers of the present invention have two
10 'monomeric' peptide units of 10 to 40 or more amino
acids, preferably 14 to about 20 amino acid residues in
length. In a preferred embodiment, these monomeric
peptide units comprise a core sequence of amino acids
X₃X₄X₅GPX₆TWX₇X₈ (SEQ ID NO: 1) where each amino acid is
indicated by standard one letter abbreviation; X₃ can be
15 C, A, α -amino- γ -bromobutyric acid, or Hoc, where Hoc is
homocysteine; X₄ can be R, H, L, or W; X₅ can be M, F,
or I; X₆ is independently selected from any one of the
20 genetically coded L-amino acids or the stereoisomeric
D-amino acids; X₇ can be D, E, I, L, or V; and X₈ can be
C, A, α -amino- γ -bromobutyric acid, or Hoc, where Hoc is
homocysteine, provided that either X₃ or X₈ is C or Hoc.
20 Preferably, the monomeric peptide unit of the dimer
comprises a core sequence YX₂X₃X₄X₅GPX₆TWX₇X₈ (SEQ ID
NO: 2) where each amino acid is indicated by standard
one letter abbreviation; each X₂ and X₈ is independently
25 selected from any one of the 20 genetically coded L-
amino acids; X₃ can be C, A, α -amino- γ -bromobutyric
acid, or Hoc, where Hoc is homocysteine; X₄ can be R, H,
L, or W; X₅ can be M, F, or I; X₇ can be D, E, I, L, or
30 V; and X₈ can be C, A, α -amino- γ -bromobutyric acid, or
Hoc, where Hoc is homocysteine, provided that either X₃
or X₈ is C or Hoc.

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1 More preferably, the monomeric peptide unit of
the dimer comprises a core sequence of amino acids
X₁YX₂X₃X₄X₅GPX₆TWX₇X₈X₉X₁₀X₁₁ (SEQ ID NO: 3), where each
5 amino acid is indicated by standard one letter
abbreviation; each X₁, X₂, X₆, X₉, X₁₀, and X₁₁ is
independently selected from any one of the 20
genetically coded L-amino acids; X₃ can be C, A,
10 α-amino-γ-bromobutyric acid, or Hoc, where Hoc is
homocysteine; X₄ can be R, H, L, or W; X₅ can be M, F,
or I; X₇ can be D, E, I, L or V; and X₈ can be C, A,
α-amino-γ-bromobutyric acid, or Hoc, where Hoc is
homocysteine, provided that either X₃ or X₈ is C or Hoc.

15 In a more preferred embodiment, both X₃ and X₈
will be C and thus, the monomeric peptide unit of the
dimer comprises a core sequence of amino acids
X₁YX₂CX₄X₅GPX₆TWX₇CX₉X₁₀X₁₁ (SEQ ID NO: 4). More
preferably, the monomeric peptide unit comprises a core
sequence of amino acids X₁YX₂CX₄X₅GPX₆TWX₇CX₉X₁₀X₁₁ (SEQ
20 ID NO: 5), where X₄ can be R or H; X₅ can be F or M; X₆
can be I, L, T, M, or V; X₇ is D or V; X₉ can be G, K,
L, Q, R, S, or T; and X₁₀ can be A, G, P, R, or Y. In a
most preferred embodiment, the monomeric peptide unit of
the dimer comprises a core sequence of amino acids
25 X₁YX₂CX₄X₅GPX₆TWX₇CX₉X₁₀X₁₁ (SEQ ID NO: 6), where X₁ can
be D, E, L, N, S, T, or V; X₂ can be A, H, K, L, M, S,
or T; X₄ is R or H; X₅ can be K, R, S, or T; and X₁₀ is
P. Particularly preferred monomeric peptide units of
the present dimers include:

30 GGLYLCRFGPVTWDCGYKGG (SEQ ID NO: 7);
GGTYSCHFGPLTWVCKPQGG (SEQ ID NO: 8);

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1 GGDYHCRMGPLTWVCKPLGG (SEQ ID NO: 9);
 VGNYMCHFGPITWVCRPGGG (SEQ ID NO: 10);
 GGVYACRMGPITWVCSPLGG (SEQ ID NO: 11);
5 VGNYMAHMGPITWVCRPGG (SEQ ID NO: 12);
 GGTYSCHFGPLTWVCKPQ (SEQ ID NO: 13);
 GGLYACHMGPMTWVCQPLRG (SEQ ID NO: 14);
 TIAQYICYMGPETWECRSPKA (SEQ ID NO: 15);
 YSCHFGPLTWVCK (SEQ ID NO: 16);
10 YCHFGPLTWVC (SEQ ID NO: 17); and
 SCHFGPLTWVCK (SEQ ID NO: 18).

15 The dimer peptides of the present invention exhibit increased biological potency in vitro and in vivo relative to the monomeric agonists from which the dimers are derived. Moreover, cell surface receptor antagonists can be 'converted' to cell surface receptor agonists in accordance with the present invention. Specifically, a cell surface receptor antagonist can be dimerized with PEG or another appropriate linker which permits mutual binding of the monomeric moieties with the receptors. As a result, the dimer exhibits effective binding to the target receptor and behaves as an agonist. Accordingly, the dimers of this invention demonstrate enhanced biological potency in vitro and in vivo relative to their monomeric forms.

20 The dimer peptides of the present invention bind to and biologically activate the cell surface receptor or otherwise behave as agonists and are preferably formed by employing polyethylene glycol as a linker between the monomeric peptide units described herein. While other conventional chemical systems can

- 14 -

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also be employed to form the dimer peptides of this invention including using other known polymeric compounds, pegylation is preferred.

5

The linking compounds of the present invention include any molecule which covalently binds the monomeric peptides at an appropriate distance or which otherwise effects dimerization of the particular cell surface receptor thereby initiating biological efficacy.

10

Starting with an appropriate synthetic peptide, containing a free amino group or other reactive site such as hydroxyls, carboxylic acids or sulfhydryls, the peptide is added in excess to a reaction mixture containing a corresponding reactive polymer. The polymer can be of a repeating nature such as polyethylene glycol, peptides, modified peptides or peptide analogs. Alternatively, the peptide can be dimerized on a small molecule scaffold such as activated benodiazepins, oxazolones, azalactones, aminimides or diketopiperazines. The most readily available linker of variable distance are ones based on linear unbranched polyethylene glycols.

15

The following is a schematic of a preferred preparatory methodology employing PEG succinimidyl propionate as the linker between the monomer units of the dimer peptides.

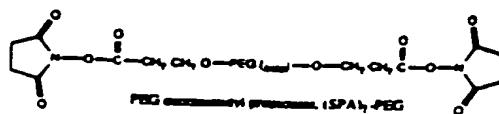
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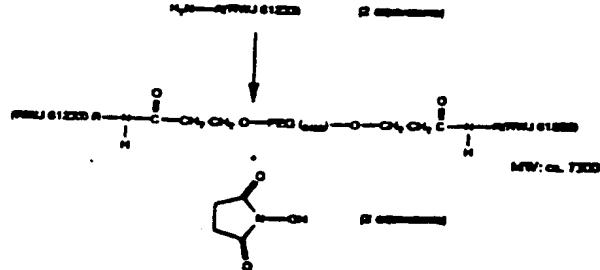
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Dimerization and especially pegylation in a head-to-head (amino to amino terminus) or head-to-tail (amino to carboxyl terminus) configuration is preferred relative to internal covalent binding of the monomeric peptides. The 'monomer' units of the dimer peptides of the present invention can be the same or different, although the same are preferred.

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The monomeric peptides which are used to form the dimers of the present invention can be prepared by classical chemical methods well known in the art. The standard methods include, for example, exclusive solid phase synthesis and recombinant DNA technology. See, e.g. Merrifield (1963) J. Am. Chem. Soc. 85:2149. Solid phase synthesis is typically commenced from the C-terminal end of the peptide using an α -amino protected resin. A suitable starting material can be prepared by attaching the required α -amino acid to a chloromethylated resin (such as BIO-BEADS SX-1, Bio Rad Laboratories, Richmond, CA), a hydroxymethyl resin, (described by Bodanszky et al. (1966) Chem. Ind.

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1 (London) 38:1597) or a benzhydrylamine resin (described by Pietta and Marshall (1970) Chem. Commun. 650).

5 The α -amino protecting groups are those known to be useful in the art of stepwise synthesis or peptides. Included are acyl type protecting groups (e.g. formyl, trifluoroacetyl, acetyl), aromatic urethane type protecting groups (e.g. benzyloxycarboyl (Cbz) and substituted Cbz), aliphatic urethane protecting groups (e.g., t-butyloxycarbonyl (Boc), isopropylloxycarbonyl, cyclohexyloxycarbonyl) and alkyl type protecting groups (e.g., benzyl and triphenylmethyl). The preferred X-amino protecting group is Fmoc. The side chain protecting group (typically ethers, esters, trityl, PMC, and the like) remains intact during coupling and is not split off during the deprotection of the amino-terminus protecting group or during coupling. The side chain protecting group must be removable upon the completion of the synthesis of the final peptide and under reaction conditions that will not alter the target peptide.

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35 The side chain protecting groups for Tyr include tetrahydropyranyl, tert-butyl, trityl, benzyl, Cbz, Z-Br-Cbz, and 2,5-dichlorobenzyl. The side chain protecting groups for Asp include benzyl, 2,6-dichlorobenzyl, methyl, ethyl, and cyclohexyl. The side chain protecting groups for Thr and Ser include acetyl, benzoyl, trityl, tetrahydropyranyl, benzyl, 2,6-dichlorobenzyl, and Cbz. The side chain protecting groups for Thr and Ser are benzyl. The side chain protecting groups for Arg include nitro, Tosy. (Tcs), Cbz, adamantyloxycarbonyl mesitoylsulfonyl (Mts), or

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1 Boc. The side chain protecting groups for Lys include
Cbz, 2-chlorobenzyloxycarbonyl (2-Cl-Cbz), 2-
bromobenzyloxycarbonyl (2-Br-Cbz), Tos, or Boc.

5 After removal of the α -amino protecting group,
the remaining protected amino acids are coupled stepwise
in the desired order. Each protected amino acid is
generally reacted in about a 3-fold excess using an
appropriate carboxyl group activator such as 2-(1H-
10 benzotriazol-1-yl)-1,1,3,3tetramethyluronium
hexafluorophosphate (HBTU) or dicyclohexylcarbodiimide
(DCC) in solution of methylene chloride (CH_2Cl_2), or
dimethyl formamide (DMF) mixtures.

15 After the desired amino acid sequence has been
completed, the desired peptide is decoupled from the
resin support by treating the mixture with a reagent
such as trifluoroacetic acid (TFA) or hydrogen fluoride
(HF). These reagents not only cleave the peptide from
the resin, but also cleave all remaining side chain
protecting groups. When the chloromethylated resin is
20 used, hydrogen fluoride treatment results in the
formation of the free peptide acids. When the
benzhydrylamine resin is used, hydrogen fluoride
treatment results directly in the free peptide amide.
Alternatively, when the chloromethylated resin is
25 employed, the side chain protected peptide can be
decoupled by treatment of the peptide resin with ammonia
to give the desired side chain protected amide or with
an alkylamine to give a side chain protected alkylamide
or dialkylamide. Side chain protection is then removed
30 in the usual fashion by treatment with hydrogen fluoride
to give the free amides, alkylamides, or dialkylamides.

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These procedures can also be used to synthesize peptides in which amino acids other than the 20 naturally occurring, genetically encoded amino acids are substituted at one, two or more positions of any of the compounds of the invention. For instance, 5 naphthylalanine can be substituted for tryptophan, facilitating synthesis. Other synthetic amino acids that can be substituted into the peptides of the present invention include L-hydroxypropyl, L-3, 10 4-dehydroxyphenylalanyl, δ amino acids such as L- δ -hydroxylysyl and D- δ -methylalanyl, L- α -methylalanyl, β amino acids, and isoquinolyl. D-amino acids and non-naturally occurring synthetic amino acids can also be 15 incorporated into the peptides of the present invention.

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In another embodiment of the present invention, a method of enhancing the in vitro or in vivo biological potency of a cell surface receptor agonist is provided. This methodology is achieved by dimerizing the receptor agonist with a linker molecule, such as 20 PEG, to form an appropriate spatial relationship between the monomeric peptide units of the dimer and thereby permitting each of the constituents of the dimers to bind to their receptors to achieve enhanced biological potency, i.e., to dimerize and thereby activate the 25 receptors to induce the relevant biological activity of the particular cell-surface receptor, e.g. EPO-R. Biological activity can be measured by the skilled artisan in various in vitro and in vivo assays and as demonstrated in the examples of the present invention.

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The peptide or molecule with binding affinity for a given receptor will have increased conformational

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1 flexibility leading to fewer barriers to effective
receptor interaction and subsequently receptor
activation. This is also indicated for molecules which
5 can bind but not activate a receptor subtype in that
such molecules can become more effective inhibitors of
ligand binding.

10 The present invention further provides a
method for altering a cell-surface receptor antagonist,
a molecule exhibiting receptor binding but no biological
activity, to behave as a cell-surface receptor agonist
15 *in vitro* or *in vivo*. This method is achieved by
dimerizing the antagonist molecule with an appropriate
linker molecule such as PEG, other polymerized molecules
or a peptide. In a preferred embodiment, an EPO
antagonist, i.e. a peptide exhibiting receptor binding
but no biological EPO activity can be altered by
dimerization to obtain a dimer which behaves as an EPO
receptor agonist. Thus, for example, in the case of
20 EPO-R these include the monomeric peptide units of the
present dimers comprising a core sequence of general
formula $(AX_2)_nX_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 19) wherein
X₂ through X₈ are as previously defined herein, in (SEQ
ID NO: 2), n is 1 or 0 and A is any one of the naturally
occurring L-amino acids except Y (tyrosine); n is
25 defined herein as the number of occurrences of (AX_2)
which can be 1 or none in the core sequence. When X₂ is
present, i.e., when n = 1, A is not tyrosine and A is
not any non-naturally occurring aromatic amino acid
analog. Such monomeric peptide units of the dimers of
30 this invention can be prepared by truncating the
peptides of Fig. 9, for example, from the N-terminus to

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1 delete the Y, tyrosine residue in SEQ ID NOS. 21 - 93.
Such monomeric peptides can also be prepared by
substitution of Y in the peptides of Fig. 9.

5 These molecules, demonstrate only binding
activity in their 'monomeric' form, but exhibit agonist
activity after dimerization with a linking compound such
as PEG. Accordingly, the present method comprises
10 identifying a monomeric peptide as herein defined which
does not demonstrate biological activity and dimerizing
that antagonist in accordance with the present invention
to obtain a cell-surface-receptor agonist i.e., in
dimeric form. Contacting the appropriate cell-surface
receptor with the thus formed dimer activates, i.e.
15 dimerizes such receptors and thus induces biological
activity of the receptor. Such monomeric units as
shown in Fig. 9 can be truncated from the N-terminus
such as SCHFGPLTWVCK (SEQ ID NO: 18) to eliminate the
tyrosine residue at position A of the formula
20 $(AX_2)_nX_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 19) or merely
substituted with any of the remaining 19 naturally
occurring amino acids or with other than a non-naturally
occurring aromatic amino acid analog. In accordance
25 with the present invention it has been determined that
the tyrosine residue at position A of the foregoing
formula is critical to biological activity of the
monomer peptide. Deletion or substitution of the
tyrosine eliminates biological activity. When dimerized
however the entity exhibits enhanced biological
activity.

30 For example, tyrosine (Y) substituted in the
formula $YX_2X_3X_4X_5GPX_6X_7$ (SEQ ID NO: 2) by p-

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1 iodohydroxyphenylalanine, p-fluorohydroxyphenylalanine,
p-amino-hydroxyphenylalanine act as EPO-R monomer
agonists but substitution with threonine or alanine for
5 tyrosine at position Y causes the monomer peptide to act
as an EPO-R antagonist. However, when dimerized in
accordance with the present invention, such dimers
behave as EPO-R agonists. The monomeric peptide units
10 identified at Fig. 9, for example, behave as EPO-R
antagonists in the absence of tyrosine at position Y of
the formula above. When such antagonists are dimerized,
the dimer behaves as an EPO-R agonist.

15 In a further embodiment of the present
invention, pharmaceutical compositions comprising at
least one of the dimers of this invention can be
employed to therapeutically treat disorders resulting
from deficiencies of biological factors such as EPO, GH,
GM-CSF, G-CSF, EGF, PDGF, VEGF, insulin, FGF and the
like. These pharmaceutical compositions may contain
20 buffers, salts and other excipients to stabilize the
composition or assist in the delivery of the dimerized
molecules.

25 In a preferred embodiment, the present
invention provides a method for treating disorders
associated with a deficiency of EPO. The method is
accomplished by administering at least one of the dimers
identified herein for a time and under conditions
sufficient to alleviate the symptoms of the disorder,
i.e. sufficient to effect dimerization or biological
activation of EPO receptors. In the case of EPO such
30 methodology is useful in the treatment of end-stage
renal failure/dialysis; anemia, especially associated

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1 with AIDS or chronic inflammatory diseases such as
rheumatoid arthritis and chronic bowel inflammation;
auto-immune disease; and for boosting the red blood cell
5 count of patient when necessary, e.g. prior to surgery
or as pretreatment to transfusion. The dimers of the
present invention which behave as EPO agonists can be
used to activate megakaryocytes.

10 Since EPO has been shown to have a mitogenic
and chemotactic effect on vascular endothelial cells as
well as an effect on central cholinergic neurons (see,
e.g., Amagnostou et al. (1990) Proc. Natl. Acad. Sci.
USA 87:597805982 and Konishi et al. (1993) Brain Res.
15 609:29-35), the compounds of this invention can also be
used to treat a variety of vascular disorders, such as
promoting wound healing, growth of collateral coronary
blood vessels (such as those that may occur after
myocardial infarction), trauma, and post vascular graft
20 treatment, and a variety of neurological disorders,
generally characterized by low absolute levels of acetyl
choline or low relative levels of acetyl choline as
compared to other neuroactive substances e.g.,
neurotransmitters.

25 Accordingly, the present invention includes
pharmaceutical compositions comprising, as an active
ingredient, at least one of the peptide dimers of the
present invention in association with a pharmaceutical
carrier or diluent. The dimers of this invention can be
administered by oral, parenteral (intramuscular,
30 intraperitoneal, intravenous (IV) or subcutaneous
injection), transdermal (either passively or using
iontophoresis or electroporation) or transmucosal

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(nasal, vaginal, rectal, or sublingual) routes of administration in dosage forms appropriate for each route of administration.

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Solid dosage forms for oral administration include capsules, tablets, pill, powders, and granules. In such solid dosage forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, or starch. Such dosage forms can also comprise, as it normal practice, additional substances other than inert diluents, e.g., lubricating, agents such as magnesium stearate. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering, agents. Tablets and pills can additionally be prepared with enteric coatings.

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Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, with the elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring and perfuming agents.

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Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting,

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1 emulsifying, and dispersing agents. They may be
sterilized by, for example, filtration through a
bacteria retaining filter, by incorporating sterilizing
5 agents into the compositions, by irradiating the
compositions, or by heating the compositions. They can
also be manufactured using sterile water, or some other
sterile injectable medium, immediately before use.

10 Compositions for rectal or vaginal
administration are preferably suppositories which may
contain, in addition to the active substance, excipients
such as cocoa butter or a suppository wax. Compositions
for nasal or sublingual administration are also prepared
with standard excipients well known in the art.

15 The dosage of active ingredient in the
compositions of this invention may be varied; however,
it is necessary that the amount of the active ingredient
shall be such that a suitable dosage form is obtained.
The selected dosage depends upon the desired therapeutic
20 effect, on the route of administration, and on the
duration of the treatment desired. Generally dosage
levels of between 0.001 to 10 mg/kg of body weight daily
are administered to mammals.

25 As can be appreciated from the disclosure
above, the present invention has a wide variety of
applications. Accordingly, the following examples are
offered by way of illustration, not by way of
limitation.

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EXAMPLE 1

SDS-PAGE gels (10-20% gradient SDS-PAGE plates, 84 x 70 x 1.0 mm, Integrated Separation Systems, Natick, MA) were stained with Coomassie Brilliant Blue R-250 (BioRad). A commercial preparation of activated difunctional polyethylene glycol (PEG-succinimidyl-propionate, SPA2, MW ca. 3400) was purchased from Shearwater Polymers, Huntsville, AL as was the monofunctional reagent, methoxy-PEG-succinimidyl-propionate, MW ca 5000. Peptide (SEQ ID NO: 8) and all other peptides were obtained from the Peptide Synthesis Facility RWJ-PRI, La Jolla, CA or Quality Controlled Biochemical, Hopkinton MA. These peptides were cyclized via oxidation of their intramolecular cysteines, amidated at the C-terminus and mass confirmed by FAB-MS. All were Ellman Reaction negative. Tris base was obtained from BioRad, Hercules, CA. (DPDPB) and trifluoracetic acid (HPLC grade) were obtained from Pierce Chemical Co., Rockford IL.

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Mono-PEG conjugation of peptide GGTYSCHFGPLTWVCKPQGG
(SEQ ID NO: 8)

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This example describes the preparation of mono-PEG conjugates of peptide (SEQ ID NO: 8), using the monofunctional amine reactive polymer analog m-SPA-PEG to be used as a control in experiments described herein. The reaction was carried out with polymer in excess (ca. 3 fold) by resuspending 142.5 mg (0.0286 mmol, MW ca. 5000) of polymer in 4 ml PBS at pH 7.5 and adding 10 mg peptide (SEQ ID NO: 8) (0.0095 mmol, MW 2092) dissolved

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1 in 1 ml of 0.1% trifluoroacetic acid. The mixture was
incubated on ice for 20 hours. The reaction was
subsequently adjusted to a final concentration 50 mM
5 Tris by the addition of 1 M tris-HCl at pH 7.5. The
reaction mixture was incubated on ice for one hour.
Analytical HPLC suggested that there were two main
reaction products of essentially equivalent magnitude
which were not baseline resolved. Preparative HPLC
10 (using the flatter gradient system described in Example
8) and conservative cuts resulted in collection of two
product peaks eluting at ca 44 and 47 minutes. After
lyophilization, 24.8 mg, 16.5 mg of each species was
recovered, respectively. Mass spectral analysis of
15 these two species demonstrated centroid masses of 7092
(peak 1) and 12036 (peak 2) indicating the coupling of
one or two PEG molecules, respectively, to the peptide
(Table I).

20 Tris inactivated polymer. Tris inactivated
polymer was formed by incubation of 5 mM SPA2 polymer
dissolved in PBS (Gibco, Gaithersburg, MD) with 50 mM
tris-HCl, pH 7.5 added and used without further
purification.

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TABLE 1
Recovery Yield of Peptide Conjugation Reaction and Apparent Molecular Mass of Product

I.D. No.	Sequence	Mass	Conjugation Reagent	Main Product Mass (centroid m/z)	Yield (% of theoretical)
8	GGTYSCHFGPPLTWCKPQCG	2092	SPA2-PEG (MW ca. 3400)	7834	69
			m-SPA-PEG (MW ca. 5000)	7092 (peak 1) 12036 (peak 2)	-
13	GGTYSCHFGPPLTWCKPKQ	1978	SPA2-PEG	7560	54
20	Ac-GGTYSCHFGPPLTWCKPQGA	2113	SPA2-PEG	7862	30
14	GGLYACHDQPRHTWVCPPLRG	2177	SPA2-PEG	7872	37
18	SCHFGPPLTWCK	1375	SPA2-PEG	6326	45

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EXAMPLE 2

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PEG dimerization of peptide GGTYSCHFGPLTWVCKPQGG
(SEQ ID NO: 8) (lot #1)

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Examples 2 - 7 describe the dimerization of various peptides described by the present invention. The modification of peptide (SEQ ID NO: 8) was carried out by resuspending 25 mg (0.0071 mmol) of polymer in 4 ml PBS at pH 7.5, and adding a 3 fold molar excess of peptide (SEQ ID NO: 8) (0.0213 mmol, 44.5 mg, MW 2092) dissolved in 1 ml of 0.1% trifluoroacetic acid. The mixture was incubated on ice. After 3 hours of incubation, an additional 7.5 mg (0.0036 mmol) of lyophilized peptide was added, resulting in a final ratio of 3.5 moles of peptide per each mole of SPA2. The mixture was incubated an additional 17 hours on ice. The reaction mixture was adjusted to a final concentration of 50 mM Tris by the addition of 1 M tris-HCl of pH 7.5 and incubated on ice for 1 hour. The sample was subjected to analytical and preparative HPLC as described in Example 8. After preparative HPLC and lyophilization, 38 mg of PEG dimer was recovered. The theoretical yield for this experiment was 55 mg based on a calculated mass of 7600 mg/mmol for a yield of 69% (Table I).

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EXAMPLE 3

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PEG dimerization of peptide GGTYSCHFGPLTWVCKPQGG
(SEQ ID NO: 8) (lot #2)

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The modification of peptide (SEQ ID NO: 8) was carried out by resuspending 25 mg (0.0071 mmol) of polymer in 4 ml PBS at pH 7.5, and adding a ca. 3 fold molar excess of peptide (SEQ ID NO: 8) (0.0213 mmol, 45.8 mg, MW 2092) dissolved in 1 ml of 0.1% trifluoroacetic acid. The mixture was incubated on ice for 22 hours. At that time, the reaction was adjusted to a final concentration of 50 mM Tris by the addition of 1 M tris-HCl, pH 7.5. The reaction mixture was incubated on ice for 1 hour. The sample was subjected to analytical and preparative HPLC as described in Example 8. After preparative HPLC and lyophilization, 37 mg of PEG dimer was recovered. The theoretical yield for this experiment was 55 mg based on a calculated mass of 7600 mg/mmol for a yield of 68% (Table I).

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EXAMPLE 4

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PEG dimerization of peptide GGTYSCHFGPLTWVCKPQ
(SEQ ID NO: 13)

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The modification of peptide (SEQ ID NO: 13) was carried out by resuspending 11.2 mg (0.0033 mmol) of polymer in 2.5 ml PBS at pH 7.5, and adding a ca. 3 fold molar excess of peptide (SEQ ID NO: 13) (0.010 mmol, 20 mg, MW 1978) dissolved in 0.25 ml of 0.1% trifluoroacetic acid. The mixture was incubated on ice for 20 hours. At that time, 0.25 ml of 1 M tris-HCl at pH 7.5 was added. The reaction mixture was incubated at 4°C for one hour. The sample was subjected to analytical and preparative HPLC as described in Example 8. The main preparative reaction product peak eluted at ca 43 minutes. After preparative HPLC and lyophilization, 13.3 mg of PEG dimer was recovered. The theoretical yield for this experiment was 24.42 mg based on a calculated mass of 7400 mg/mmol for a yield of 54% (Table I).

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EXAMPLE 5

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PEG dimerization of peptide Ac-GGTY SCHFGPLTWVCKPQGG
(SEQ ID NO: 20)

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The modification of peptide (SEQ ID NO: 20) was carried out by resuspending 10.5 mg (0.0031 mmol) of polymer in 2.5 ml PBS at pH 7.5, and adding a ca. 3 fold molar excess of peptide (SEQ ID NO: 20) (0.0094 mmol, 20 mg, MW 2133) dissolved in 0.25 ml of 0.1% trifluoroacetic acid and the mixture incubated at 4°C for 28 hours. At that time, the reaction as monitored by HPLC was estimated to be approximately 30% complete, the temperature was shifted to ambient and an additional 27 hour incubation provided no net increase in product. Because of possible hydrolysis of the reactive polymer, an additional 5 mg of polymer was added and the incubation was continued for an additional 16 hours. At that time, 0.25 ml of 1 M tris-HCl, pH 7.5 was added and the reaction mix was incubated at 4°C for an additional 1 hour. The sample was subjected to analytical and preparative HPLC using a flatter gradient system as described in Example 8. The main preparative reaction product peak eluting at ca 48 minutes. After preparative HPLC and lyophilization, 10.4 mg of PEG dimer was recovered. The theoretical yield for this experiment was 34.4 mg based on a calculated mass of 7650 mg/mmol for a yield of 30% (Table I).

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EXAMPLE 6PEG dimerization of peptide (SEQ ID NO: 14)

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The modification of peptide (SEQ ID NO: 14) was carried out by resuspending 2.6 mg (0.00076 mmol) of polymer in 3.0 ml PBS at pH 7.5 and adding a ca. 3 fold molar excess of peptide (SEQ ID NO: 14) (0.00229 mmol, 5 mg, MW 2177) dissolved in 0.1 ml of 0.1% trifluoroacetic acid. The mixture was incubated on ice for 26 hours. At that time, 0.25 ml of 1 M-tris-HCl at pH 7.5 was added. The reaction mixture was incubated at 4°C for 1 hour. The sample was subjected to analytical and preparative HPLC using the flatter gradient system described in Example 8. The main preparative reaction product peak eluted at ca 46 minutes. After preparative HPLC and lyophilization, 2.2 mg of PEG dimer was recovered. The theoretical yield for this experiment was 24.42 mg based on a calculated mass of 7400 mg/mmol for a yield of 37% (Table I).

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EXAMPLE 9

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This example demonstrates the interaction of peptide (SEQ ID NO: 8), peptide (SEQ ID NO: 16), peptide (SEQ ID NO: 18) and peptide (SEQ ID NO: 13) with EPO binding protein (EBP) using a bifunctional sulphhydryl reactive crosslinker, (1,4-Di-[3'-(2'-pyridyldithio)propionamido]butane DPDPB.

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To explore the interaction of peptide (SEQ ID NO: 8) with EBP, a bifunctional sulphhydryl reactive crosslinker (DPDPB) was used in an attempt to stabilize a mimetic dependent dimeric structure. Control experiments demonstrated that the crosslinker does not inactivate the EPO binding potential of EBP or the proliferative properties of peptide (SEQ ID NO: 8). As shown in Figure 4, a dimeric EBP product was formed by co-incubation of the peptide, peptide (SEQ ID NO: 8), DPDPB and EBP. This data shows the ability of the peptide (SEQ ID NO: 8) to mediate formation of a soluble receptor dimer. To further explore this question, peptides (SEQ ID NO: 13), (SEQ ID NO: 16) and (SEQ ID NO: 18) were examined for their ability to mediate dimerization. As shown in Figure 4, lanes 7A and 8A, when peptide (SEQ ID NO: 13) was truncated at the carboxyl terminus, it retained good in vitro bioactivity and improved in vivo bioactivity, resulting in a crosslinking signal similar to peptide (SEQ ID NO: 8). However, peptide (SEQ ID NO: 18) did not appear to stabilize the dimerization signal (Figure 4, lanes 9A

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and 10A) whereas peptide (SEQ ID NO: 16) (Figure 4, lanes 5A and 6A) gave a strong dimerization band. These two peptides differ by a single N-terminal tyrosine residue and display a similar profile in the in vitro proliferation assay with peptide (SEQ ID NO: 18) being inactive. Peptide (SEQ ID NO: 16) has an ED₅₀ of 3 μ M on murine receptor cells. Both peptides have similar IC₅₀ values indicating that they both retain binding activity. These results demonstrate that EBP dimerization is a property of the EPO peptide series and that the presence of the tyrosine is critical for this activity and that this corresponds to in vitro bioactivity.

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1 minutes. The major product peak eluting at 48 minutes
was collected and lyophilized (Figure 2). These elution
conditions were subsequently modified to improve the
5 resolution of some conjugation products peptide (SEQ ID
NO: 20), mPEG-peptide (SEQ ID NO: 8), peptide (SEQ ID
NO: 14) from reaction by products. This was
accomplished by application of a flatter linear gradient
10 of 20-80% B over 60 minutes. The variation in retention
time due to different peptides and elution condition is
described as part of each synthesis example. The
materials recovered from the main product peak from each
15 reaction were subsequently analyzed by analytical
reverse phase HPLC, MALDI-TOF mass spectrometry, EPO
competitive binding potential and for in vitro
bioactivity.

20 The activated PEG used in these experiments
has an approximate molecular weight of 3400 and has
amine reactive succinimidyl groups on either end of the
difunctional linear polymer. This reactivity was
employed to couple two equivalents of peptide (SEQ ID
25 NO: 8) (MW= 2092) to the polymer with the concomitant
liberation of two succinimidyl moieties resulting in a
dimeric product as shown in Scheme I. Peptide (SEQ ID
NO: 8) contains two potentially reactive amines, one at
the N-terminus of the peptide and one in the side chain
25 of the single lysine within the peptide sequence, so
that a number of different connectivities between the
two molecules was possible.

30 MALDI-TOF mass spectral analysis was
supportive of the presence of the expected dimeric
product (Figure 3) as indicated by a predominant species.

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with a centroid mass of 7661. This data shows that the dimeric product described in the present invention was produced using the methods described herein.

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EXAMPLE 7

PEG dimerization of peptide (SEQ ID NO: 18)

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The modification of peptide (SEQ ID NO: 18) was carried out by resuspending 1.2 mg (0.00036 mmol) of polymer in 0.5 ml PBS at pH 7.5, and adding a ca. 3 fold molar excess of the peptide (0.0011 mmol, 1.5 mg, MW 2177) dissolved in 0.05 ml of 0.1% trifluoroacetic acid.

10

The mixture was incubated on ice for 20 hours. At that time, 0.1 ml of 1 M tris-HCl at pH 7.5 was added. The reaction mixture was incubated at 4°C for 1 hour. The sample was subjected to purification using an analytical HPLC system as described in Example 8. The main reaction product peak eluted at ca 38 minutes. After preparative HPLC and lyophilization, 1 mg of PEG dimer was recovered. The theoretical yield for this experiment was 2.2 mg based on a calculated mass of 6150 mg/mmol for a yield of 45% (Table I).

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EXAMPLE 8

5 The accumulation of the dimers described above
in Examples 1-7 was monitored by analytical reverse
phase HPLC. The analysis was carried out using a Vydac
C-18 Protein-Peptide column (0.46 x 25 cm, part no.
10 218TP54) and a Rainin Gradient HPLC system fitted with a
Dynamax dual wavelength detector. At injection, the
column was equilibrated in 0.1% TFA in dH₂O and was
developed with a 45 minute linear gradient (0-100%) of
15 acetonitrile (ACN) containing 0.1% TFA beginning at 10
minutes after injection. The flow rate was held
constant at 1 ml/min. Under these analytical
conditions, the SPA2 polymer and tris inactivated
polymer did not appear to bind the column while a major
reaction product with a retention time (37 minutes) was
20 identified (Figure 1). Peptide (SEQ ID NO: 8)
demonstrated a retention time of 35 minutes and the
excess peptide utilized in the reaction was clearly
distinguished from the nascent reaction products.

25 The main product reaction product peak was
purified by preparative reverse phase HPLC on the same
chromatographic system using a Vydac C-18 Protein-
Peptide column (2.2 x 25 cm, part no. 218TP15022).
Injection of the reaction mix (6 ml) occurred with the
30 column equilibrated at 80:20, H₂O:ACN (both containing
0.1% TFA) at a constant flow rate of 8 ml/min. After a
20 minute wash, the column was developed by application
of a linear gradient of 100% ACN/0/1% TFA over 60

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EXAMPLE 10

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IMMOBILIZED EBP BASED [¹²⁵I]EPO COMPETITION BINDING

ASSAY

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This study examined the binding capacity of the EPO PEG dimers to bind EPO receptors.

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The extracellular domain of the human erythropoietin receptor (EPO binding protein, EBP) was expressed and overproduced in E. coli. As with many other recombinant eukaryotic proteins produced in E. coli, the protein appeared as an insoluble product in laboratory scale fermentations and was refolded and purified to obtain active protein. EPO binding protein produced by this method contains one free sulphhydryl group which can be modified without effecting the solution phase binding of ligand. In order to immobilize the EPO binding protein for equilibrium binding analysis and for competition binding assay, the EPO binding protein was covalently attached to agarose beads.

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The iodoacetyl activation chemistry of Sufolink beads (Pierce Chemical Co, Rockford, IL) is specific for free thiols and assures that the linkage is not easily reversible. EBP-Sufolink beads were made as follows: SulfoLink gel suspension (10 ml) was mixed with of coupling buffer (40 ml: 50 mM Tris, pH 8.3, 5 mM EDTA) and the gel was allowed to settle. The supernatant was removed and the EPO binding protein (0.3-1 mg/ml in coupling buffer) to be bound was added directly to the washed beads. The mixture was rocked

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1 gently for 30 minutes at room temperature and the beads
were allowed to settle for 1 hour at room temperature.
The supernatant was removed and retained. The beads
5 were washed twice with 20 ml of coupling buffer. The
washes were recovered as well. The beads were then
treated with 20 ml of 0.05 M cysteine for 30 minutes at
room temperature to block unbound sites. Finally, the
beads were washed with 50 ml of 1 M NaCl, then with 30
10 ml of PBS, and resuspended in 20 ml of PBS and stored at
4°C. The amount of EBP which was covalently bound to
the beads was determined by comparing the OD₂₈₀ of the
original EBP solution to the total OD₂₈₀ recovered in
the reaction supernatant and the two 20 ml washes.
15 Typically, 40-60% of the applied EBP remains associated
with the beads.

Binding assays were initiated by the addition
of EPO binding protein beads (50 μ l) to individual
reaction tubes. Total binding was measured in tubes
20 containing 0.3-30 nM [¹²⁵I]EPO (NEN Research Products,
Boston MA, 100 μ Ci/ μ g). For determination of
non-specific binding, unlabelled EPO was added at a
level of 1000 fold in excess of the corresponding
[¹²⁵I]EPO concentration. Each reaction volume was
25 brought to 500 μ l with binding buffer (PBS/0.2% BSA).
The tubes were incubated for five hours (a time period
experimentally determined as adequate for the
establishment of equilibrium) at room temperature with
gentle rocking. After five hours, each reaction mixture
30 was passed through a 1 ml pipet tip plugged with glass
wool. The tubes were washed with 1 ml wash buffer (PBS/
5% BSA) and this volume as well as 2 additional 1 ml

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1 washes were passed through the pipet tip and collected
for determination of the free EPO concentration.
Equilibrium binding analysis of the specific association
5 of [¹²⁵I]EPO with EPO mimetic binding proteins
immobilized on these agarose beads indicates a Kd of 5
nm ± 2 based on a linear transformation (Scatchard) of
the binding isotherm (Figure 5).

10 Competitive binding analysis assays of
candidate peptides and dimer peptides were performed as
outlined below. Individual peptides were dissolved in
DMSO to prepare a stock solution 1 mM. Dimer peptides
were contained within PBS at a concentration of 5 mM.
15 All reaction tubes (in duplicate) contained 50 µL of EBP
beads, 0.5 nM [¹²⁵I]EPO and 0-500 µM peptide in a total
of 500 µL binding buffer.

20 The final concentration of DMSO was adjusted
to 2.5% in all peptide assay tubes. At this
concentration DMSO has no detectable effect since an
examination of the sensitivity of the assay to DMSO
demonstrated that concentrations of up to 25% DMSO (V/V)
had no deleterious effect on binding. Non-specific
25 binding was measured in each individual assay by
inclusion of tubes containing a large excess of
unlabelled EPO (1000 nM). Initial assay points with no
added peptide were included in each assay to determine
total binding. Binding mixtures were incubated
overnight at room temperature with gentle rocking. The
beads were then collected using Micro-columns (Isolab,
30 Inc.) and washed with 3 mL of wash buffer. The columns
containing the washed beads were placed in 12 x 75 mm
glass tubes and bound radioactivity levels determined in

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1 a gamma counter. The amount of bound [¹²⁵I]EPO was
expressed as a percentage of the control (total=100%)
binding and plotted versus the peptide concentration
5 after correction for non-specific binding. The IC₅₀ was
defined as the concentration of the analyte which
reduced the binding of [¹²⁵I]EPO to the EBP beads by
50%. All data are reported as relative to peptide (SEQ
ID NO: 8) which demonstrated an IC₅₀ of 5 μ M.

10 Competitive binding analysis revealed an IC₅₀
of 20 μ M for the purified dimer, a value four fold
greater than peptide (SEQ ID NO: 8) in the same assay
(Figure 5 and Table II). Polymer alone, which was
15 inactivated by treatment with Tris-HCl, demonstrated a
detectable competition binding signal but this signal
was modest (<10%) at the IC₅₀ of the PEG-peptide (SEQ ID
NO: 8) dimer.

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TABLE II

Table II. Binding and Cell Proliferation Studies

Id	Relative Binding*	EPO-ED ₅₀ (μM)*	
		mouse receptor	transfected human receptor
D. No. 8	1	0.1	0.09
ris inact. polymer	60	IA2	IA
q. I.D. No. 8 covalent dimer #1	4	0.01 (10X)	0.0015 (60X)
q. I.D. No. 8 covalent dimer #2	3	0.01 (10X)	0.002 (45X)
D. No. 13	1.6	0.08	0.02
sq. I.D. No. 13 covalent dimer	3	0.01 (8X)	0.002 (10X)
D. No. 20 (N-acetyl)	4	0.03	0.06
sq. I.D. No. 20 covalent dimer	12	0.2 (-7X)	0.05
D. No. 14 (terminal NH ₂)	0.6	0.1	0.08
sq. I.D. No. 14 covalent dimer	-	0.006 (16X)	0.001 (80X)

* Amino acid required to achieve the half maximal level of EPO-dependent proliferation (11 μM)
 Binding relative to Seq. I.D. No. 8
 2Aminocaproic
 Note that all peptides are cyclic and were analyzed as COOH-terminal amides (-CONH₂)

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EXAMPLE 11

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This example shows the improved potency of PEG-EPO peptide dimers to EPO receptors in human and murine cell lines.

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Cell line FDC-P1/ER, an EPO-dependent line expressing the murine EPO receptor, was grown and maintained as described previously (Carroll et al. 1991). Also employed was cell line FDC-P1/trER expressing a functional truncated human EPO receptor (missing the C-terminal 40 amino acids). Both cell lines exhibit EPO dependent cellular proliferation. Briefly, cells were maintained in RPMI 1640 media (Gibco/BRL) containing 10% heat-inactivated fetal calf serum and 10 units/ml of recombinant human EPO. For the cellular proliferation assay, FDC-P1/ER or FDC-P1/trER cells were grown to stationary phase, centrifuged, washed with RPMI 1640 media (no EPO), and plated in EPO minus media for 24 hr.

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After 24 hours, the cells were counted, resuspended at 800,000 cells/ml and dispensed at 40,000 cells/well. Stock solutions of the peptide dimer (5 mM in PBS) and peptide (10 mM in DMSO) were prepared and dispensed in triplicate to final concentrations of 1 x 10⁻¹⁰ M through 1 x 10⁻⁵ M and adjusted to a final volume of 0.2 ml. Final DMSO concentrations of 0.1% (V/V, maximal) or less were found to have no cellular toxicity or stimulatory effects. A standard EPO dose response curve was generated with each assay series.

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1 After a 42 hr incubation at 37°C (ca. 2 cell doublings)
1 μ Ci/well of [3 H] thymidine was added and the incubation
continued for 6 hr at which time the cells were
5 harvested and counted to assess [3 H]thymidine
incorporation as a measure of cell proliferation.
Results are expressed as the amount of peptide or dimer
peptide necessary to yield one half of the maximal
activity obtained with recombinant EPO.

10 As shown in Figure 5 and Table II, the initial
lot of PEG-peptide (SEQ ID NO: 8) dimer demonstrated
ED₅₀ values of 0.01 μ m and 0.0015 μ m in EPO responsive
cell lines containing the murine or human EPO receptor,
respectively. In both cell lines, the parent peptide,
15 peptide (SEQ ID NO: 8), demonstrated an ED₅₀ of 0.1 μ M,
indicating an increase in potency of 10 fold in the
murine receptor line and almost 60 fold in the human
receptor containing cells. Thus, the dimer was clearly
more potent in murine and human lines than the peptides
themselves. This was confirmed by generation of a
20 second synthesis lot of PEG-peptide (SEQ ID NO: 8) dimer
which resulted in a 10 and 45 fold increase in potency
in the murine and human lines, respectively. Polymer
alone, which was inactivated by treatment with Tris-HCl,
demonstrated no activity in the cell proliferation
25 assay.

30 A second EPO mimetic peptide, peptide (SEQ ID
NO: 13), with the sequence GGTYSCHFGPLTWVCKPQ, was also
subjected to a similar PEG dimerization protocol as that
described above for peptide (SEQ ID NO: 8). The dimer
product of PEG-peptide (SEQ ID NO: 13) is also more
active than the unconjugated parent compound (Table II).

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1 Both of these dimer peptides have ultimate ED₅₀ values
near 0.002μM. In spite of this more modest increase,
the experimental evidence clearly indicates that the
5 dimerization of these peptides with PEG results in
improved potency.

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EXAMPLE 12

5 To further examine the connectivity of the peptides of the present invention to PEG, peptide molecules, which contained only an internal lysine group were used peptide (SEQ ID NO: 8) analog acetylated at the N-terminus peptide (SEQ ID NO: 20) and a sequence analog peptide (SEQ ID NO: 14) which only had a reactive N-terminal amine were PEG dimerized. In vitro
10 proliferation data of these compounds suggest that potential dimerization through the free amino terminus has the most profound effect on bioactivity giving rise to a species about 80 fold more active than the monomeric parent peptide (SEQ ID NO: 14) dimer.
15 Conjugation through the lysine side chain had no real effect on activity peptide (SEQ ID NO: 20) as did mono-PEG or di-PEG conjugation (Table III). This data indicates that the creation of a head to head dimer
20 (both peptides attached through the N-terminus) using a PEG linker greatly enhances the potency of EPO peptides and approaches a level almost two logs greater than the free parent peptide. Further, this effect was not observed upon simple covalent attachment of linear PEG to peptide (SEQ ID NO: 8) indicating that dimerization
25 is a critical determinant for this increased activity.

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TABLE III

Table III. Binding and Cell Proliferation Studies mPEG/

Compound	Relative Binding*	EPO-ED ₅₀ (μM)*	
		mouse receptor	truncated human receptor
Seq. I.D. No. 8	1	0.1	0.09
mPEG/Seq. I.D. No. 8, peak #1	~60	2	0.1
mPEG/Seq. I.D. No. 8, peak #2	>40	1	0.4

* Amounts required to achieve the half maximal level of EPO dependent proliferation (11pM)
 1 ND=Not determined
 2 Active
 3 Binding relative to Seq. I.D. No. 8

Note that all peptides are cyclic and were analyzed as COOH terminal amine (-CONH₂)

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EXAMPLE 13

Polycythemic Exhypoxic Mouse Bioassay.

5 This study demonstrates the ability of peptide (SEQ ID NO: 8)/PEG-dimers to retain in vivo bioactivity. Peptides were assayed for in vivo activity in the polycythemic mouse bioassay adapted from the method described by Cotes and Bangham (1961), Nature 191: 1065-1067. BDF1 mice were allowed to acclimate to ambient conditions for 7-10 days. Body weights were determined for all animals. Low weight animals (<15 grams) were not used. Mice were introduced to hypobaric chambers with a 24 hour conditioning cycle consisting of 0.40% +/- 0.02 atm. for 18 hours followed by 6 hours at ambient pressure for a total of 14 days. Following the 14 day period, mice were placed in ambient pressure for 72 hours prior to dosing. Test samples or recombinant Human Erythropoietin (rHuEPO) standards were diluted in an assay vehicle consisting of Phosphate Buffered Saline (PBS)-0.1% Bovine Serum Albumin (BSA). Peptide sample stock solutions (excluding peptide dimers) were first solubilized in dimethyl sulfoxide (DMSO). Control groups included one group of vehicle alone, and one group of (DMSO) at final concentration of 1%.

10 Each dose group contained 10 mice. Mice were injected subcutaneously (scruff of neck) with 0.5 ml of the appropriate sample. Forty eight hours following the sample injection, the mice were administered an intraperitoneal injection of 0.2 ml of [⁵⁵Fe]

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1 (approximately 18.0 milliCuries/milligram, Dupont, NEN)
and 0.75 microCuries/Mouse.

5 Mouse body weights were determined twenty four hours following [⁵⁹Fe] administration and the mice were sacrificed forty eight hours following the [⁵⁹Fe] injection. Blood was collected from each animal by cardiac puncture and hematocrits were determined (heparin was used as the anticoagulant). Each blood sample (0.2 ml) was analyzed for [⁵⁹Fe] incorporation using a Packard gamma counter. Non-responder mice (i.e., those mice with radioactive incorporation less than the negative control group) were eliminated from the appropriate data set. Mice that had hematocrit values less than 53% were also eliminated.

10 15 This assay examined the ability of an exogenously administered compound to induce new red blood cell synthesis, or in other words to function as EPO or an EPO-mimetic. The results are derived from sets of 10 animals for each experimental dose. As shown in Figure 7 and Table IV, the data suggests that on a mole equivalent basis, peptide (SEQ ID NO: 8)/PEG-dimer is about 10 fold more active than peptide (SEQ ID NO: 8) monomer. These results are consistent with in vitro results in which increased potency values of 10 fold was observed on murine EPO-R bearing cells.

TABLE IV

30 Table 4. Experimetic Mouse Bioassay Study of PEG Dimer Activity

Compound	Amount required for equivalence to 0.025U EPO (nmol) n=10
Seq. I.D. No. 8	3.8

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EXAMPLE 14

This example shows that an inactive truncation analog of peptide (SEQ ID NO: 8), which lacks the critical tyrosine peptide (SEQ ID NO: 18), (SCHFGPLTWVCK), can be converted to an agonist on the human EPO receptor cell line by PEG dimerization. In this experiment, a 10^{-5} M concentration of the parent peptide had no activity above background while the dimeric peptide exhibited a level of proliferation twice as many cpm as background. As shown in Figure 8, the peptide alone (open squares) did not induce proliferation of the EPO responsive cells but upon PEG dimerization (open diamonds) a significant agonist effect was observed. Approximately twice as many cpm incorporated over non-stimulated cells at 10^{-5} M added peptide dimer. The replicate error bars represent the standard deviation of three assay points per concentration of peptide or peptide dimer.

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1 Exhypoxic polycythemic mouse bioassay:

5 PEG dimer and the monomer parent peptide RWJ 61718 were compared in the exhypoxic mouse bioassay (Table 1). This peptide exhibited an 80 fold increase in in vitro activity upon dimerization. Murine studies (in vivo) of the activity of the dimer compared to the monomer peptide revealed a 250 fold increase in activity of RWJ 61718 upon dimerization. Cellular proliferation studies on this dimer peptide in murine receptor containing cells demonstrated a 16 fold increase over the monomer indicating that the 250 fold increase

10 in vivo might be attributable to other factors such as altered metabolism or prolonged circulatory half-life which occur upon PEG dimerization of the peptide sequence. Thus, in addition to the effect of dimerization alone, the PEG modification has an effect which impacts in vivo activity and may be specific to individual peptide sequences.

15

Cell associated EPO receptor competition binding assay.

20 A competitive binding analysis of the ability of selected monomer peptides and their cognate dimer products to compete with radiolabelled EPO binding for cell associated human EPO receptors was performed Erythropoietin Receptor Competition Binding Analysis was preformed as follows. TF-1 cells were maintained in RPMI 1640, 10% fetal calf serum, 1% L-glutamine, 1% penicillin, 0.1% streptomycin and 1 ng/ml of GM-CSF. [125]-EPO was obtained from NEN Research Products. Cells were centrifuged and washed 1 x with binding buffer (RPMI 1640, 5% BSA, 25 mM Hepes, pH 7.5, 0.02% sodium azide) resuspended in binding buffer, and counted using trypan blue as an indicator of viability. Each reaction contained approximately 5×10^5 cells, [125]-EPO (0.5 nM), no competitor or peptide or dimer preparation in a final volume of 200 μ l. The binding reactions (in duplicate) were incubated overnight at 4°C. Following binding, the tubes were centrifuged at 12,000 rpm for 1 min at 4°C in a

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1 refrigerated centrifuge. The supernatant was removed, the cell
5 pellet resuspended in 100 μ l of binding buffer, and the cell
suspension layered onto 0.7 ml of bovine calf serum. The tubes were
10 centrifuged at 12,000 rpm for 5 min at 4°C, the supernatant was
removed, the bottom of the tubes snipped off, and the cell pellets
15 counted in a Micromedic ME plus gamma counter. Non-specific binding
was determined by incubating cells with [¹²⁵]EPO and a 100-fold
excess of non-radioactive EPO. These data demonstrate increases in
20 apparent binding competitive affinity of 3.0 fold, 3.2 fold and 80
fold for peptides RWJ 61233, RWJ 61596 and RWJ 61718, respectively
(Table 2). In vivo proliferation studies with these peptides and
25 their dimer derivatives reveal increases in potency of ea. 50 fold,
10 fold and 80 fold, respectively, indicating that the magnitude of
increased binding affinity is exceeded by the functional potency of
the peptide for two of the three species. Thus, the effect of
30 dimerization and subsequent increase in activity may be one in which
the efficiency of receptor stimulation is improved by limiting the
lateral diffusion of the receptors away from a binding event.
Peptide dimerization therefore likely results in entropic rather
than enthalpic gains upon mimetic ligand-receptor association for
some peptide dimer sequences.

Unlike the EBP-bead EPO competitive binding assay where peptide
dimerization negatively impacted the ability of PEG dimer peptides
to compete for receptor binding, the ability to compete for cell
25 associated receptors is increased by dimerization. This may be due
to the ability of the cell associated receptor to dimerize while the
immobilized EBP monomer likely cannot.

30 Conversion of inactive to active peptide RWJ 61177 was further
studied. An improved and expanded study was performed which
confirmed our earlier observation of conversion to an active peptide
(Figure 6, Panel D).

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TABLE IV. EXHYPOXIC MOUSE BIOASSAY STUDY OF PEG DIMER ACTIVITY

Compound	Amount required for equivalency to 0.025 U EPO (nmol) n=10
RWJ 61233 (seq. ID #8)	3.8
SAP2/61233, covalent dimer	0.28
RWJ 61718 (seq. ID #14)	18
SAP2/61718, covalent dimer	0.07

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TABLE V. EPO COMPETITIVE BINDING ANALYSIS OF CELL ASSOCIATED RECEPTORS

Compound	IC ₅₀ (μM)
RWJ 61233 (seq ID #8)	3.8
SAP2/61233, covalent dimer	0.28
RWJ 61718 (seq ID#14)	18
SAP2/61718, covalent dimer	0.07

SEQUENCE LISTING

GENERAL INFORMATION:

i) APPLICANT: Johnson, Dana L
Zivin, Robert A

.i) TITLE OF INVENTION: AGONIST PEPTIDE DIMERS

.i) NUMBER OF SEQUENCES: 93

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(F) ZIP: 11530

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/484,135
(B) FILING DATE: 07-JUN-1995
(C) CLASSIFICATION:

.ii) ATTORNEY/AGENT INFORMATION:

(A) NAME: DiGiglio, Frank S
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(C) REFERENCE/DOCKET NUMBER: 9594

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (516) 742-4343
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INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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c) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..10
- (D) OTHER INFORMATION: /note= "Xaa(Pos1) can be C,A, α -amino- γ -bromobutyric acid or Hoc; Xaa(Pos2) can be R,H,L or W; Xaa(Pos3) can be M,F or I; Xaa(Pos6) can be any one of the 20 L-amino acids or the stereoisomeric D-amino acids; Xaa(Pos9) can be D,E,I,L or V; and Xaa(Pos10) can be C,A, α -amino- γ -bromobutyric acid or Hoc, provided that either Xaa(Pos1) or Xaa(Pos10) is C or Hoc"

i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Xaa Xaa Xaa Gly Pro Xaa Thr Trp Xaa Xaa
5 10

INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "Xaa(Pos2) and Xaa(Pos8) can be any one of the 20 L-amino acids; Xaa(Pos3) can be C,A, α -amino- γ -bromobutyric acid or Hoc; Xaa(Pos4) can be R,H,L or W; Xaa(Pos5) can be M,F or I; Xaa(Pos11) can be D,E,I,L or V; and Xaa(Pos12) can be C,A, α -amino- γ -bromobutyric acid or Hoc, provided that either Xaa(Pos3) or Xaa(Pos12) is C or Hoc"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Tyr Xaa Xaa Xaa Xaa Gly Pro Xaa Thr Trp Xaa Xaa
1 5 10

INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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;) FEATURE:

(A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /note= "Xaa(Pos1), Xaa(Pos 3), Xaa(Pos9), Xaa(Pos14), Xaa(Pos15) and Xaa(Pos16) can be any one of 20 L-amino acids; Xaa(Pos4) can be C,A, α -amino- γ -bromobutyric acid or Hoc; Xaa(Pos5) can be R,H,L or W; Xaa(Pos6) can be M,F or I; Xaa(Pos12) can be D,E,I,L or V; and Xaa(Pos13) can be C,A, α -amino- γ -bromobutyric acid or Hoc, provided that either Xaa(Pos4) or Xaa(Pos13) is C or Hoc"

i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

aa Tyr Xaa Xaa Xaa Xaa Gly Pro Xaa Thr Trp Xaa Xaa Xaa Xaa Xaa Xaa
5 10 15

INFORMATION FOR SEQ ID NO:4:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Xaa Tyr Xaa Cys Xaa Xaa Gly Pro Xaa Thr Trp Xaa Cys Xaa Xaa Xaa
 1 5 10 15

INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /note= "Xaa(Pos1), Xaa(Pos3) and Xaa(Pos16) can be any one of the 20 L-amino acids; Xaa(Pos5) can be R or H; Xaa(Pos6) can be F or M; Xaa(Pos9) can be I,L,T,M or V; Xaa(Pos12) can be D or V; Xaa(Pos14) can be C,W,I,Q,R,S or T; Xaa(Pos15) can be A,G,P,R or Y"

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i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

aa Tyr Xaa Cys Xaa Xaa Gly Pro Xaa Thr Trp Xaa Cys Xaa Xaa Xaa
5 10 15

FORMATION FOR SEQ ID NO:6:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..16
- (D) OTHER INFORMATION: /note= "Xaa(Pos1) can be D,E,L,N,S,T or V; Xaa(Pos3) can be A,H,K,L,M,S or T; Xaa(Pos5) can be R or H; Xaa(Pos6) can be F or M; Xaa(Pos9) can be I,L,T,M or V; Xaa(Pos12) can be D or V; Xaa(Pos14) can be K,R,S or T; Xaa(Pos15) is P and Xaa(Pos16) can be any one of the 20 L-amino acids"

xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Xaa Tyr Xaa Cys Xaa Xaa Gly Pro Xaa Thr Trp Xaa Cys Xaa Xaa Xaa
1 5 10 15

FORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Gly Leu Tyr Leu Cys Arg Phe Gly Pro Val Thr Trp Asp Cys Gly
1 5 10 15

Tyr Lys Gly Gly
20

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FORMATION FOR SEQ ID NO:8:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

iii) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ly Gly Thr Tyr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys
5 10 15

ro Gln Gly Gly
20

FORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gly Gly Asp Tyr His Cys Arg Met Gly Pro Leu Thr Trp Val Cys Lys
1 5 10 15

Pro Leu Gly Gly
20

FORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Val Gly Asn Tyr Met Cys His Phe Gly Pro Ile Thr Trp Val Cys Arg
1 5 10 15

Pro Gly Gly
20

INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Gly Val Tyr Ala Cys Arg Met Gly Pro Ile Thr Trp Val Cys Ser
1 5 10 15

Pro Leu Gly Gly
20

INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Val Gly Asn Tyr Met Ala His Met Gly Pro Ile Thr Trp Val Cys Arg
1 5 10 15

Pro Gly Gly

INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- i) MOLECULE TYPE: peptide

- i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ly Gly Thr Tyr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys
5 10 15

ro Gln

FORMATION FOR SEQ ID NO:14:

- i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- ii) MOLECULE TYPE: peptide

- xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly Gly Leu Tyr Ala Cys His Met Gly Pro Met Thr Trp Val Cys Gln
1 5 10 15

Pro Leu Arg Gly
20

FORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Thr Ile Ala Gln Tyr Ile Cys Tyr Met Gly Pro Glu Thr Trp Glu Cys
1 5 10 15

Arg Pro Ser Pro Lys Ala
20

FORMATION FOR SEQ ID NO:16:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

iii) SEQUENCE DESCRIPTION: SEQ ID NO:16:

hr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys
5 10

FORMATION FOR SEQ ID NO:17:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Tyr Cys His Phe Gly Pro Leu Thr Trp Val Cys
1 5 10

FORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys
1 5 10

INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids

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- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

c) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "Xaa(Pos1) can be any one of the 20 L-amino acids; except that Xaa(Pos1) may or may not be Y and Xaa(Pos1) may be any non-naturally occurring aromatic acid analog when Xaa(Pos1) is Y. Xaa(Pos2) and Xaa(Pos8) can be any one of the 20 L-amino acids; Xaa(Pos3) can be C,A, α -amino- γ -bromobutyric acid or Hoc; Xaa(Pos4) can be R,H,L or W; Xaa(Pos5) can be M,F or I; Xaa(Pos11) can be D,E,I,L or V and Xaa(Pos12) can be C,A, α -amino- γ -bromobutyric acid or Hoc provided that either Xaa(Pos3) or Xaa(Pos12) is C or Hoc"

:i) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Xaa Xaa Xaa Xaa Xaa Gly Pro Xaa Thr Trp Xaa Xaa
5 10

INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gly Gly Thr Tyr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys
1 5 10 15

Pro Gln Gly Gly
20

INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

ii) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ly Gly Thr Tyr Arg Cys Ser Met Gly Pro Met Thr Trp Val Cys Leu
5 10 15
ro Met Gly Gly
20

FORMATION FOR SEQ ID NO:22:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gly Gly Met Tyr Ser Cys Arg Met Gly Pro Met Thr Trp Val Cys Gly
1 5 10 15
Pro Ser Gly Gly
20

FORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gly Gly Trp Ala Trp Cys Arg Met Gly Pro Ile Thr Trp Val Cys Ser
1 5 10 15
Ala His Gly Gly
20

FORMATION FOR SEQ ID NO:24:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gly Gly Met Tyr Ser Cys Arg Met Gly Pro Met Thr Trp Val Cys Ile
1 5 10 15

Pro Tyr Gly Gly
20

INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Gly Gly Glu Tyr Lys Cys Tyr Met Gly Pro Ile Thr Trp Val Cys Lys
1 5 10 15

Pro Glu Gly Gly
20

INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ly Gly Asp Tyr Thr Cys Arg Met Gly Pro Met Thr Trp Ile Cys Thr
5 10 15

la Thr Gly Gly
20

INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Gly Gly Asn Tyr Leu Cys Arg Phe Gly Pro Gly Thr Trp Asp Cys Thr
1 5 10 15

Gly Phe Arg Gly
20

INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Gly Gly Asn Tyr Val Cys Arg Met Gly Pro Ile Thr Trp Ile Cys Thr
1 5 10 15

Pro Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gly Lys Asp Val Cys Arg Met Gly Pro Ile Thr Trp Asp Cys Arg
5 10 15
Ser Thr Gly Gly
20

INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gly Gly Ser Tyr Leu Cys Arg Met Gly Pro Thr Thr Trp Leu Cys Thr
1 5 10 15
Ala Gln Arg Gly Gly Gly Asn
20

INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Gly Gly Asn Tyr Leu Cys Arg Met Gly Pro Ala Thr Trp Val Cys Gly
1 5 10 15
Arg Met Gly Gly
20

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FORMATION FOR SEQ ID NO:32:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

.i) MOLECULE TYPE: peptide

:i) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Gly Gly Glu Tyr Lys Cys Arg Met Gly Pro Leu Thr Trp Val Cys Gln
1 5 10 15

Tyr Ala Gly Gly
20

NFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Gly Gly Asp Tyr Thr Cys Arg Met Gly Pro Met Thr Trp Ile Cys Thr
1 5 10 15

Ala Thr Arg Gly
20

INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(i) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Gly Gly Val Tyr Val Cys Arg Met Gly Pro Leu Thr Trp Glu Cys Thr
1 5 10 15
Ala Ser Gly Gly
20

INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Gly Gly Glu Tyr Ser Cys Arg Met Gly Pro Met Thr Trp Val Cys Ser
1 5 10 15
Pro Thr Gly Gly
20

INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Gly Gly Glu Tyr Leu Cys Arg Met Gly Pro Ile Thr Trp Val Cys Glu
1 5 10 15
Arg Tyr Gly Gly
20

INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

ii) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ly Gly Asn Tyr Ile Cys Arg Met Gly Pro Met Thr Trp Val Cys Thr
5 10 15
la His Gly Gly
20

INFORMATION FOR SEQ ID NO:38:

- i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Gly Gly Asp Tyr Leu Cys Arg Met Gly Pro Ala Thr Trp Val Cys Gly
1 5 10 15
Arg Met Gly Gly
20

INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Gly Gly Leu Tyr Leu Cys Arg Phe Gly Pro Val Thr Trp Asp Cys Gly
1 5 10 15
Tyr Lys Gly Gly
20

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FORMATION FOR SEQ ID NO:40:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:40:

ly Gly Leu Tyr Ser Cys Arg Met Gly Pro Ile Thr Trp Val Cys Thr
5 10 15

ys Ala Gly Gly
20

FORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Gly Gly Gly Tyr His Cys Arg Met Gly Pro Met Thr Trp Val Cys Arg
1 5 10 15

Pro Val Gly Gly
20

FORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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) SEQUENCE DESCRIPTION: SEQ ID NO:42:

y Gly Thr Tyr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys
5 10 15
o Gln Gly Gly
20

FORMATION FOR SEQ ID NO:43:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

iii) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Gly Gly Ile Tyr Lys Cys Leu Met Gly Pro Leu Thr Trp Val Cys Thr
5 10 15
Pro Asp Gly Gly
20

FORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Gly Gly Leu Tyr Ser Cys Leu Met Gly Pro Ile Thr Trp Leu Cys Lys
1 5 10 15
Pro Lys Gly Gly
20

FORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION: SEQ ID NO:45:

Gly Asp Tyr His Cys Arg Met Gly Pro Leu Thr Trp Val Cys Lys
5 10 15

Leu Gly Gly
20

FORMATION FOR SEQ ID NO:46:

- i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:46:

ly Gly Asp Tyr Ser Cys Arg Met Gly Pro Thr Thr Trp Val Cys Thr
5 10 15

ro Pro Gly Gly
20

FORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Gly Gly Asp Tyr Trp Cys Arg Met Gly Pro Ser Thr Trp Glu Cys Asn
1 5 10 15

Ala His Gly Gly
20

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FORMATION FOR SEQ ID NO:48:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

.i) MOLECULE TYPE: peptide

ci) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Gly Gly Lys Tyr Leu Cys Ser Phe Gly Pro Ile Thr Trp Val Cys Ala
1 5 10 15

Arg Tyr Gly Gly
20

NFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Gly Gly Leu Tyr Lys Cys Arg Leu Gly Pro Ile Thr Trp Val Cys Ser
1 5 10 15

Pro Leu Gly Gly
20

INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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i) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Gly Gly Ser Tyr Thr Cys Arg Phe Gly Pro Glu Thr Trp Val Cys Arg
5 10 15

Pro Asn Gly Gly
20

INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Gly Gly Ser Tyr Ser Cys Arg Met Gly Pro Ile Thr Trp Val Cys Lys
1 5 10 15

Pro Gly Gly Gly
20

INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Gly Gly Ser Tyr Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Leu
1 5 10 15

Pro Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

ii) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Gly Gly Leu Tyr Glu Cys Arg Met Gly Pro Met Thr Trp Val Cys Arg
1 5 10 15
Pro Gly Gly Gly
20

INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Gly Gly Asp Tyr Thr Cys Arg Met Gly Pro Ile Thr Trp Ile Cys Thr
1 5 10 15
Lys Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Gly Gly Val Tyr Ser Cys Arg Met Gly Pro Thr Thr Trp Glu Cys Asn
1 5 10 15
Arg Tyr Val Gly
20

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INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Gly Gly Ala Tyr Leu Cys His Met Gly Pro Ile Thr Trp Val Cys Arg
1 5 10 15

Pro Gln Gly Gly
20

INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Gly Gly Glu Tyr Ser Cys Arg Met Gly Pro Asn Thr Trp Val Cys Lys
1 5 10 15

Pro Val Gly Gly
20

INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:61:

ly Gly Val Tyr Lys Cys Arg Met Gly Pro Leu Thr Trp Glu Cys Arg
5 10 15

ro Thr Gly Gly
20

INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Gly Gly Asp Tyr Asn Cys Arg Phe Gly Pro Leu Thr Trp Val Cys Lys
1 5 10 15

Pro Ser Gly Gly
20

INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Gly Gly Ser Tyr Leu Cys Arg Phe Gly Pro Thr Thr Trp Leu Cys Ser
1 5 10 15

Ser Ala Gly Gly
20

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i) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Gly Leu Tyr Leu Cys Arg Met Gly Pro Val Thr Trp Glu Cys Gln
5 10 15

Pro Arg Gly Gly
20

FORMATION FOR SEQ ID NO:59:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

.i) MOLECULE TYPE: peptide

.ii) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Gly Gly Leu Tyr Thr Cys Pro Met Gly Pro Ile Thr Trp Val Cys Leu
1 5 10 15

Leu Pro Gly Gly
20

INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Gly Gly Leu Tyr Thr Cys Arg Met Gly Pro Val Thr Trp Val Cys Thr
1 5 10 15

Gly Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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i) SEQUENCE DESCRIPTION: SEQ ID NO:66:

ly Gly Trp Val Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Gly
5 10 15

al His Gly Gly
20

INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Gly Gly Gln Leu Leu Cys Gly Ile Gly Pro Ile Thr Trp Val Cys Arg
1 5 10 15

Trp Val Gly Gly
20

INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Gly Gly Lys Tyr Ser Cys Phe Met Gly Pro Thr Thr Trp Val Cys Ser
1 5 10 15

Pro Val Gly Arg Gly Val
20

INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION: SEQ ID NO:77:

Gly Leu Tyr Leu Cys Arg Met Gly Pro Gln Thr Trp Met Cys Gln
5 10 15

Gly Gly Gly
20

FORMATION FOR SEQ ID NO:78:

- i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Gly Asp Tyr Val Cys Arg Met Gly Pro Met Thr Trp Val Cys Ala
5 10 15

Tyr Gly Arg
20

FORMATION FOR SEQ ID NO:79:

- ii) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

ii) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Gly Gly Trp Tyr Ser Cys Leu Met Gly Pro Met Thr Trp Val Cys Lys
1 5 10 15

Ala His Arg Gly
20

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FORMATION FOR SEQ ID NO:80:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Gly Gly Lys Tyr Tyr Cys Trp Met Gly Pro Met Thr Trp Val Cys Ser
1 5 10 15

Pro Ala Gly Gly
20

FORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Gly Gly Tyr Val Met Cys Arg Ile Gly Pro Ile Thr Trp Val Cys Asp
1 5 10 15

Ile Pro Gly Gly
20

INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

— 84 —

i) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Gly Ser Cys Leu Gln Cys Cys Ile Gly Pro Ile Thr Trp Val Cys Arg
5 10 15
Lys Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

Gly Gly Asn Tyr Phe Cys Arg Met Gly Pro Ile Thr Trp Val Cys Gln
1 5 10 15
Arg Ser Val Gly
20

INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

Gly Gly Glu Tyr Ile Cys Arg Met Gly Pro Leu Thr Trp Glu Cys Lys
1 5 10 15
Arg Thr Gly Gly
20

INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

- 85 -

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Gly Gly Leu Tyr Ala Cys Arg Met Gly Pro Ile Thr Trp Val Cys Lys
1 5 10 15

Tyr Met Ala Gly
20

INFORMATION FOR SEQ ID NO:86:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Gly Gly Gln Tyr Leu Cys Thr Phe Gly Pro Ile Thr Trp Leu Cys Arg
1 5 10 15

Gly Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:87:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Gly Gly Val Tyr Ala Cys Arg Met Gly Pro Ile Thr Trp Val Cys Ser
1 5 10 15

Pro Leu Gly Gly
20

- 86 -

INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

Gly Gly Tyr Thr Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Ser
1 5 10 15

Ala His Gly Gly
20

INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Gly Gly Thr Tyr Lys Cys Trp Met Gly Pro Met Thr Trp Val Cys Arg
1 5 10 15

Pro Val Gly Gly
20

INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- 87 -

i.) SEQUENCE DESCRIPTION: SEQ ID NO:90:

Ily Gly Asn Tyr Tyr Cys Arg Phe Gly Pro Ile Thr Phe Glu Cys His
5 10 15
ro Thr Gly Gly
20

FORMATION FOR SEQ ID NO:91:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i.) MOLECULE TYPE: peptide

ii.) SEQUENCE DESCRIPTION: SEQ ID NO:91:

Ily Gly Glu Tyr Leu Cys Arg Met Gly Pro Asn Thr Trp Val Cys Thr
1 5 10 15
Pro Val Gly Gly
20

INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii.) MOLECULE TYPE: peptide

xi.) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Gly Gly Leu Tyr Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Leu
1 5 10 15
Pro Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

- 88 -

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Gly Gly Leu Tyr Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Leu
1 5 10 15

Pro Ala Gly Gly
20

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Gly Gly Trp Val Tyr Cys Arg Ile Gly Pro Ile Thr Trp Val Cys Asp
1 5 10 15

Thr Asn Gly Gly
20

INFORMATION FOR SEQ ID NO:70:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Gly Gly Met Tyr Tyr Cys Arg Met Gly Pro Met Thr Trp Val Cys Lys
1 5 10 15

Gly Ala Gly Gly
20

INFORMATION FOR SEQ ID NO:71:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Gly Gly Thr Thr Gln Cys Trp Ile Gly Pro Ile Thr Trp Val Cys Arg
1 5 10 15

Ala Arg Gly Gly
20

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INFORMATION FOR SEQ ID NO:72:

- i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- ii) MOLECULE TYPE: peptide

.i) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Gly Gly Pro Tyr His Cys Arg Met Gly Pro Ile Thr Trp Val Cys Gly
5 10 15

Pro Val Gly Gly
20

INFORMATION FOR SEQ ID NO:73:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Gly Gly Glu Tyr Arg Cys Arg Met Gly Pro Ile Ser Trp Val Cys Ser
1 5 10 15

Pro Gln Gly Gly
20

INFORMATION FOR SEQ ID NO:74:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

- 91 -

) SEQUENCE DESCRIPTION: SEQ ID NO:74:

y Gly Asn Tyr Thr Cys Arg Phe Gly Pro Leu Thr Trp Glu Cys Thr
5 10 15

o Gln Gly Gly Ala
20

FORMATION FOR SEQ ID NO:75:

.) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:75:

ly Gly Ser Trp Asp Cys Arg Ile Gly Pro Ile Thr Trp Val Cys Lys
5 10 15

rp Ser Gly Gly
20

FORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Val Gly Asn Tyr Met Cys His Phe Gly Pro Ile Thr Trp Val Cys Arg
1 5 10 15

Pro Gly Gly Gly
20

FORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

WE CLAIM:

1. A. peptide dimer comprising two monomeric peptides of 10 to 5 about 40 amino acids in length that bind to EPO receptor, each monomeric peptide comprising a sequence of amino acids $X_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 1) wherein X_6 is selected from any of the 20 genetically coded L-amino acids; X_1 is C; X_3 is R, H, L or W; X_4 is M, F or I; X_5 is D, E, I, L or V; and X_8 is C.
10
2. The peptide dimer of Claim 1 wherein each of said monomeric peptides comprise a sequence of amino acids $YX_2X_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 2) wherein each of X_2 and X_6 is independently selected from any one of the 20 genetically coded L-amino acids; 15 X_3 is C; X_4 is R, H, L or W; X_5 is M, F or I; X_7 is D, E, I, L or V; and X_8 is C.
3. The peptide dimer of Claim 2 wherein each of said monomeric peptides comprise a sequence of amino acids 20 $X_1YX_2X_3X_4X_5GPX_6TWX_7X_8X_9X_{10}X_{11}$ (SEQ ID NO: 3) wherein each of X_1 , X_2 , X_6 , X_9 , X_{10} , and X_{11} is independently selected from any one of the 20 genetically coded L-amino acids; X_3 is C; X_4 is R, H, L or W; X_5 is M, F or I; X_7 is D, E, I, L or V; and X_8 is C.
- 25 4. The peptide dimer of Claim 3 wherein X_4 , is R or H; X_5 is F or M; X_6 is I, L, T, M or V; X_7 is D or V; X_9 is G, K, L, Q, R, S, or T; and X_{10} is A, G, P, R, or Y.
- 30 5. The peptide dimer of Claim 4 wherein X_1 is D, E, L, N, S, T or V; X_2 is A, H, K, L, M, S, or T; X_4 is R or H; X_9 is K, R, S, or T; and X_{10} is P.
6. The peptide dimer of Claim 1 wherein said monomeric peptides are

	GGLYLCRFGPVTWDCGYKGG	(SEQ ID NO: 7);
	GGTYSCHFGPLTWVCKPQGG	(SEQ ID NO: 8);
	GGDYHCRMGPLTWVCKPLGG	(SEQ ID NO: 9);
	VGNYMCHFGPITWVCRPGGG	(SEQ ID NO: 10);
5	GGVYACRMGPITWVCSPPLGG	(SEQ ID NO: 11);
	VGNYMAHMGPITWVCRPGGG	(SEQ ID NO: 12);
	GGTYSCHFGPLTWVCKPQ	(SEQ ID NO: 13);
	GGLYACHMGPMTWVCQPLRG	(SEQ ID NO: 14);
	TIAQYICYMGPETWECRSPKA	(SEQ ID NO: 15);
10	YSCHFGPLTWVCK	(SEQ ID NO: 16);
	YCHFGPLTWVC	(SEQ ID NO: 17); and
	SCHFGPLTWVCK	(SEQ ID NO: 18)

7. A pharmaceutical composition comprising at least one peptide dimer of any one of Claims 1-6.

8. A method for treating a patient having a disorder characterized by a deficiency of EPO or low or defective red blood cell population comprising administering to said patient a therapeutically effective amount of at least one peptide dimer of any one of Claims 1-6.

9. The peptide dimer of any one of Claims 1-6 wherein said dimer is formed by a polyethylene glycol linker through a covalent bond.

10. The peptide dimer of any one of Claims 1-6 wherein said monomeric peptide units are dimerized on activated benodiazepins, oxazalones, azalactones, aminimides or diketopiperazine.

11. The peptide dimer of Claim 9 wherein said monomeric peptides are covalently bound N-terminus to N-terminus.

12. The peptide dimer of Claim 10 wherein said monomeric peptides are covalently bound N-terminus to N-terminus.

13. The peptide dimer of Claim 9 wherein said monomeric peptides are covalently bound N-terminus to C-terminus.

14. The peptide of Claim 10 wherein said monomeric peptides are covalently bound N-terminus to C-terminus.

10 15. A method of improving the bioactivity of a cell surface receptor comprising dimerizing a monomeric agonist of said cell surface receptor and contacting said formed dimer with said cell surface receptor to effect said improved biological activity.

15 16. A method of activating a cell surface receptor to induce biological activity of said cell surface receptor comprising dimerizing a monomeric agonist of said cell surface receptor and contacting said formed dimer with said receptor thereby inducing said biological activity.

20

17. The method of Claim 15 or 16 wherein said cell surface receptor is contacted with said dimer in vitro or in vivo.

25 18. The method of Claim 15 or 16 wherein said cell surface receptor is EPO-R.

30 19. The method of Claim 15 or 16 wherein said cell surface agonist is a GH agonist, PDGF agonist, EGF agonist, G-CSF agonist, TPO agonist, VEGF agonist, FGF agonist, insulin agonist, IL-3 agonist, IL-5 agonist, IL-6 agonist or IL-2 agonist.

20. The method of Claim 15 or 16 wherein said agonist comprises a sequence of amino acids YX₂X₃X₄X₅GPX₆TWX₇X₈ (SEQ ID NO: 2)

wherein each of X_2 and X_6 is independently selected from any one of the 20 genetically coded L-amino acids; X_3 is C; X_4 is R, H, L or W; X_5 is M, F or I; X_7 is D, E, I, L or V; and X_8 is C.

5 21. The method of Claim 15 or 16 wherein said agonist comprises a sequence of amino acids $X_1YX_2X_3X_4X_5GPX_6TWX_7X_8X_9X_{10}X_{11}$ (SEQ ID NO: 3) wherein each of X_1 , X_2 , X_6 , X_9 , X_{10} , and X_{11} is independently selected from any one of the 20 genetically coded L-amino acids; X_3 is C; X_4 is R, H, L or W; X_5 is M, F or I; X_7 is D, E, I, L or 10 V; and X_8 is C.

22. The method of Claim 15 or 16 wherein said agonist comprises a sequence of amino acids $X_1YX_2X_3X_4X_5GPX_6TWX_7X_8X_9X_{10}X_{11}$ (SEQ ID NO: 3) wherein each of X_1 , X_2 , and X_{11} , is independently selected from 15 any one of the 20 genetically coded L-amino acids; X_3 is C; X_4 is R or H; X_5 is F or M; X_6 is I, L, T, M or V; X_7 is D or V; X_8 is G, K, L, Q, R, S, or T; and X_{10} is A, G, P, R, or Y.

23. The method of Claims 15 or 16 wherein said agonist 20 comprises a sequence of amino acids $X_1YX_2X_3X_4X_5GPX_6TWX_7X_8X_9X_{10}X_{11}$ (SEQ ID NO: 3) wherein X_1 is D, E, L, N, S, T or V; X_2 is A, H, K, L, M, S, or T; X_3 is C; X_4 is R or H; X_5 is M, F or I; X_6 and X_{11} are independently any one of the 20 genetically coded L-amino acids; X_7 is D, E, I, L or V; X_8 is C; X_9 is K, R, S, or T; 25 and X_{10} is P.

24. The method of Claim 15 or 16 wherein said agonist is selected from the group consisting of:

30 GGLYLCRFGPVTWDCGYKGG (SEQ ID NO: 7);
GGTYSCHFGPLTWVCKPQGG (SEQ ID NO: 8);
GGDYHCRMGPPLTWVCKPLGG (SEQ ID NO: 9);
VGNYMCHFGPITWVCRPGGG (SEQ ID NO: 10);
GGVYACRMGPITWVCSPLGG (SEQ ID NO: 11);

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VGNYMAHMGPITWVCRPGG	(SEQ	ID	NO:	12);
GGTYSCHFGPLTWVCKPQ	(SEQ	ID	NO:	13);
GGLYACHMGPMTWVCQPLRG	(SEQ	ID	NO:	14);
TIAQYICYMGPETWECRSPSKA	(SEQ	ID	NO:	15);
YSCHFGPLTWVCK	(SEQ	ID	NO:	16); and
YCHFGPLTWVC	(SEQ	ID	NO:	17).

25. The method of Claim 15 or 16 wherein said peptide dimers
are formed with a polyethylene glycol linker through a covalent
10 bond.

26. A method of preparing a cell surface receptor agonist comprising dimerizing a cell surface antagonist.

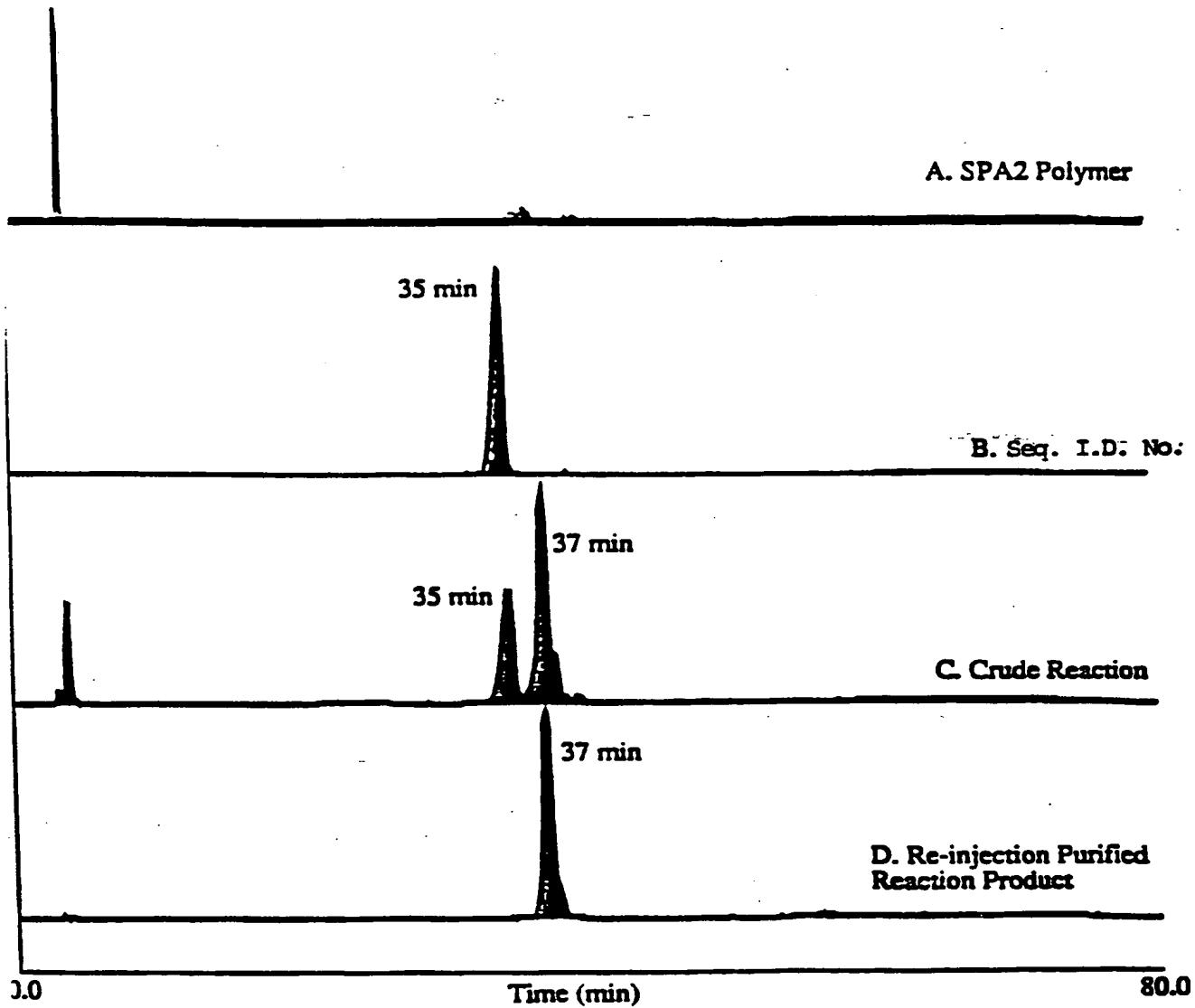
15 27. The method of Claim 26 wherein said cell surface antagonist
receptor is a GH antagonist, PDGF antagonist, EGF antagonist, G-
CSF antagonist, EGF antagonist, GM-CSF antagonist, TPO
antagonist, VEGF antagonist, FGF antagonist, insulin antagonist,
IL-3 antagonist, IL-5 antagonist, IL-6 antagonist, or an IL-2
20 antagonist.

28. The method of Claim 26 wherein said cell surface receptor antagonist is a EPO-R antagonist.

25 29. The method of Claim 28 wherein said antagonist comprises a sequence of amino acids $(AX_2)_nX_3X_4X_5GPX_6TWX_7X_8$ (SEQ ID NO: 19) wherein X_6 is selected from any of the 20 genetically coded L-amino acids; X_3 is C; X_4 is R, H, L or W; X_5 , is M, F or I; X_7 , is D, E, I, L or V; X_8 is C; X_2 is selected from any of the 20 genetically coded L-amino acids, n is 0 or 1 and A is any of the 20 genetically coded L-amino acids except Y (tyrosine).
30

30. The method of Claim 21 where said antagonist is SCHFGPLTWVCK (SEQ ID NO: 18).

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Reverse Phase Analysis of SPA2 Reaction with SEQ. I.D. NO. 8**Figure 1**

Preparative Reverse Phase Analysis of PEG- Peptide (SEQ. I.D. No. 8) Dimer

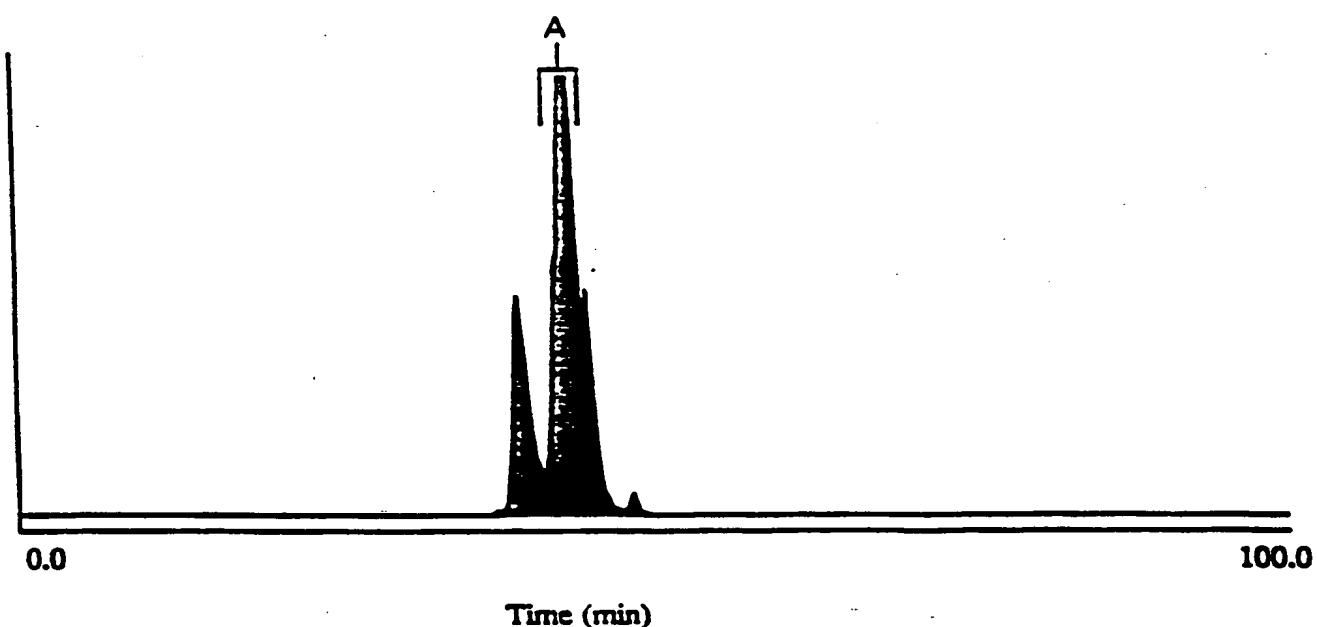
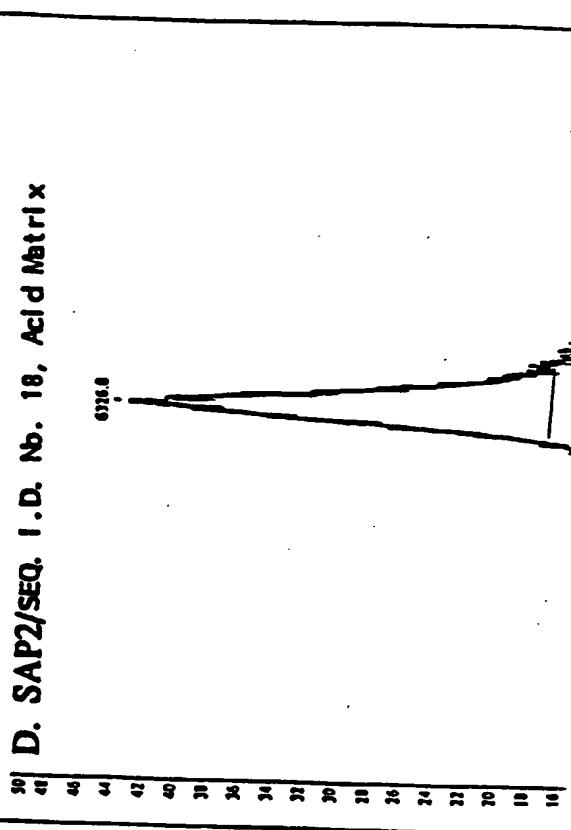
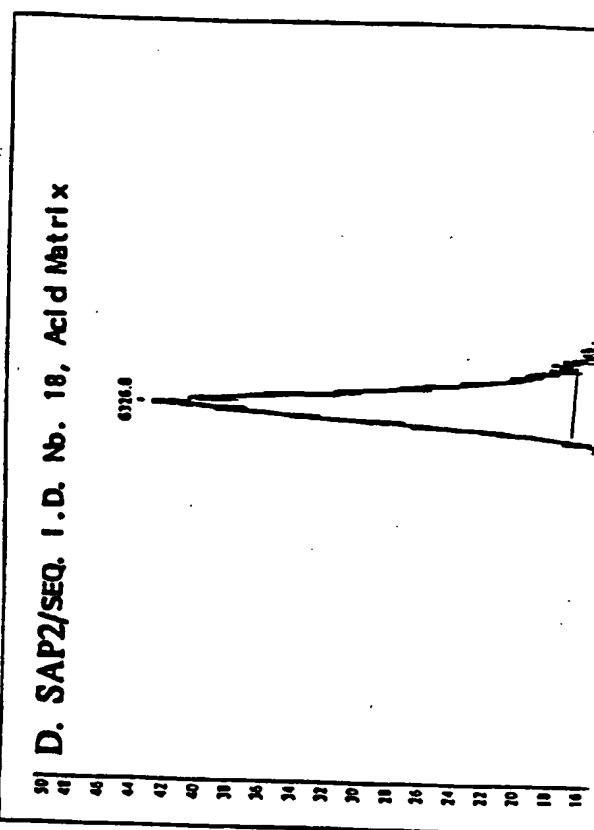
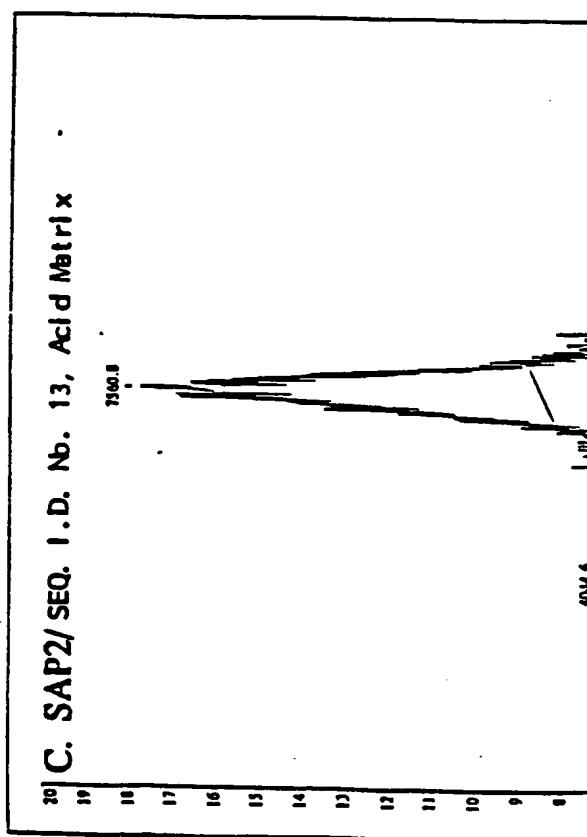
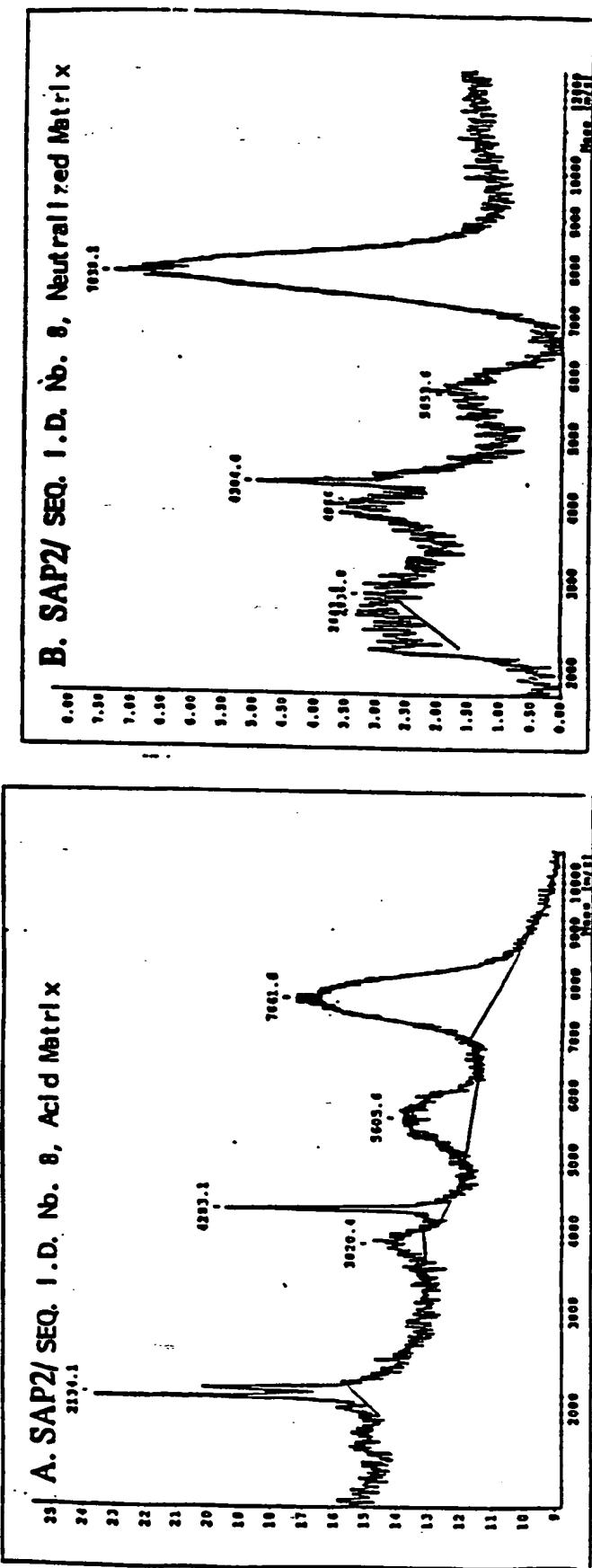
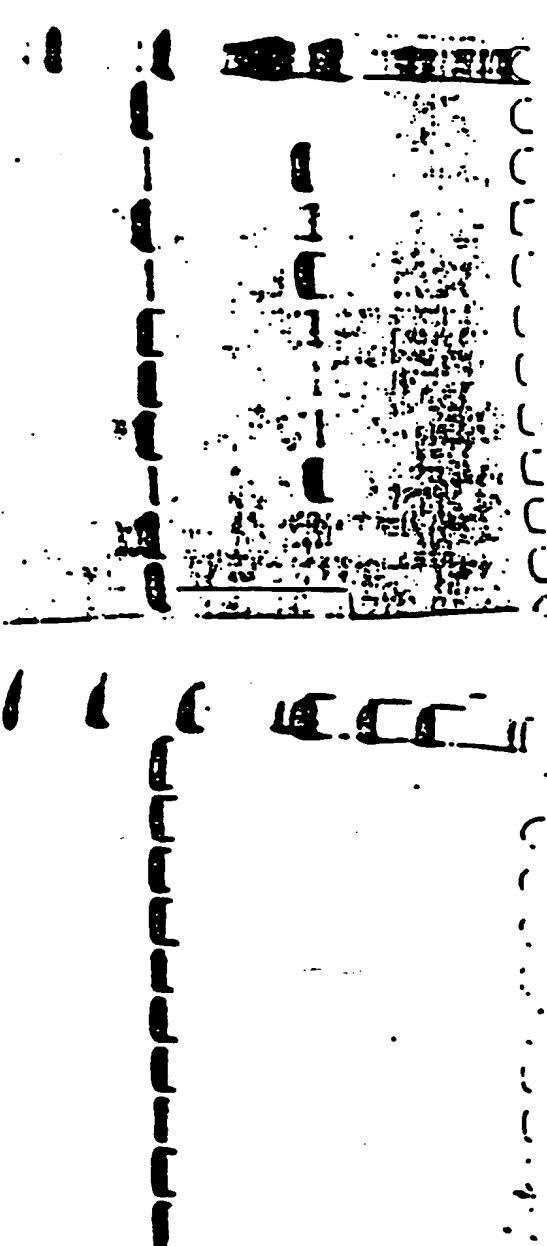


Figure 2

Figure 3



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A. Non Reducing 10-20% SDS-PAGE, B. Reducing 10-20% SDS-PAGE

Lane	Seq. 1 .D. Nb.	8	16	18	13
1		2	3	4	5
MWM	EBP	Peptide (μM)	400	400	400
	EBP (μM)		22	22	22
DPCP (mM)		1.1	0	1.1	0

Seq. 1 .D. Nb.	Sequence	IC ₅₀ (μM)	EP0-ED ₅₀ (μM)
8	GGTYSCHFGPLTMVCKPQGG	5	0.1
16	YSCHFGPLTMVCK	70	3
18	SCHFGPLTMVCK	90	IA
13	GGTYSCHFGPLTMVCKPQ	6	0.08

Figure 4

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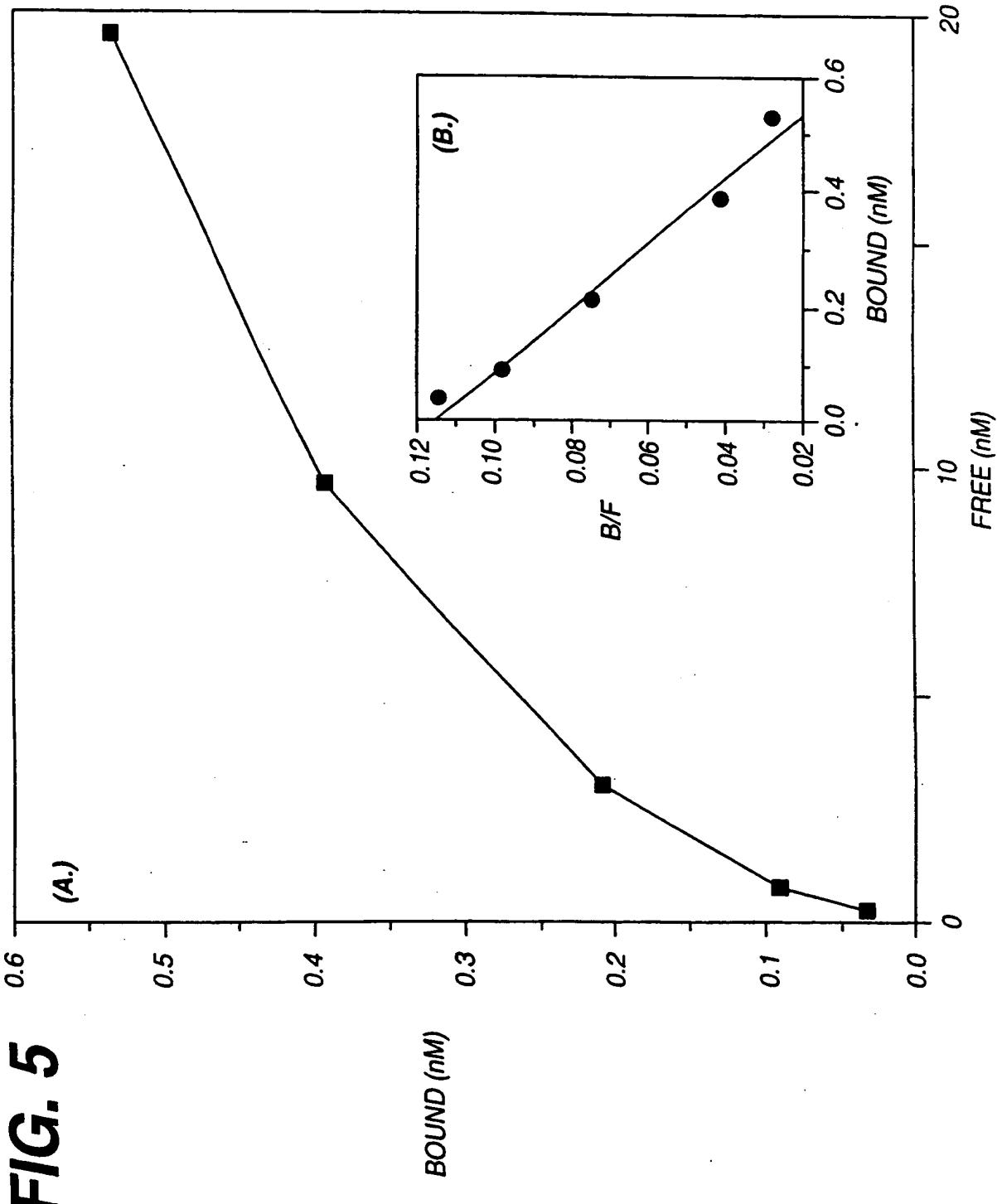
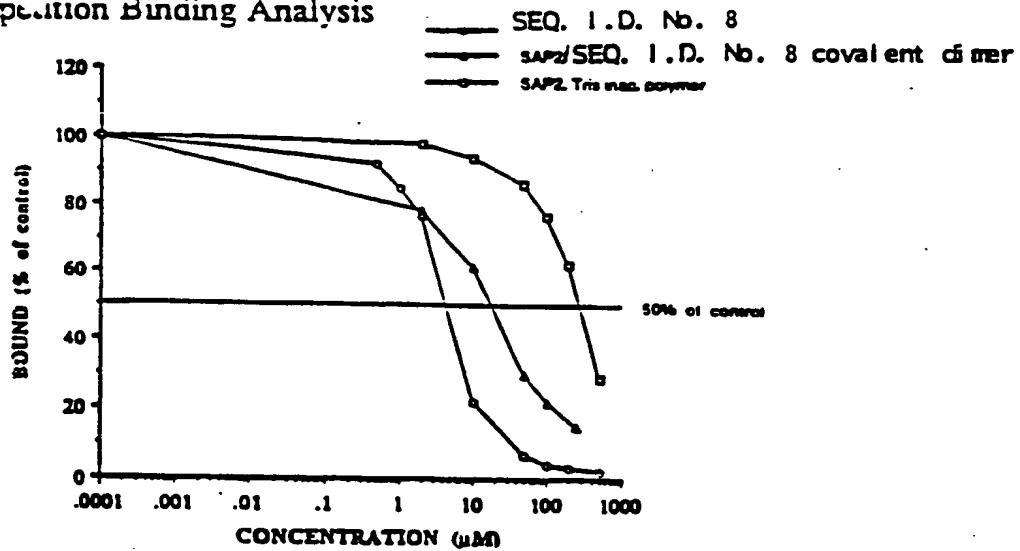
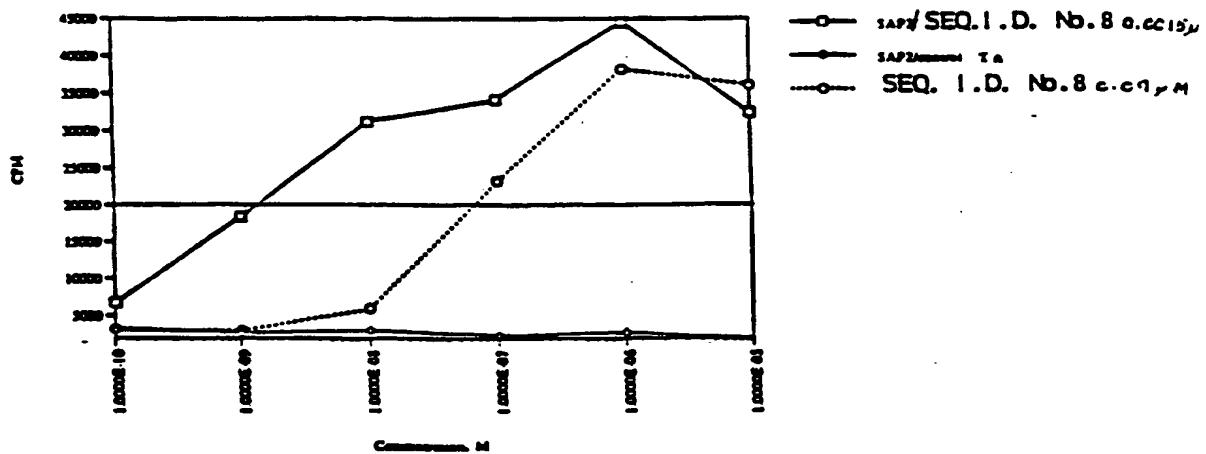


FIG. 5

A. [¹²⁵I]EPO Competition Binding Analysis

B. Cell Proliferation, Human EPOR



C. Cell Proliferation, Murine EPOR

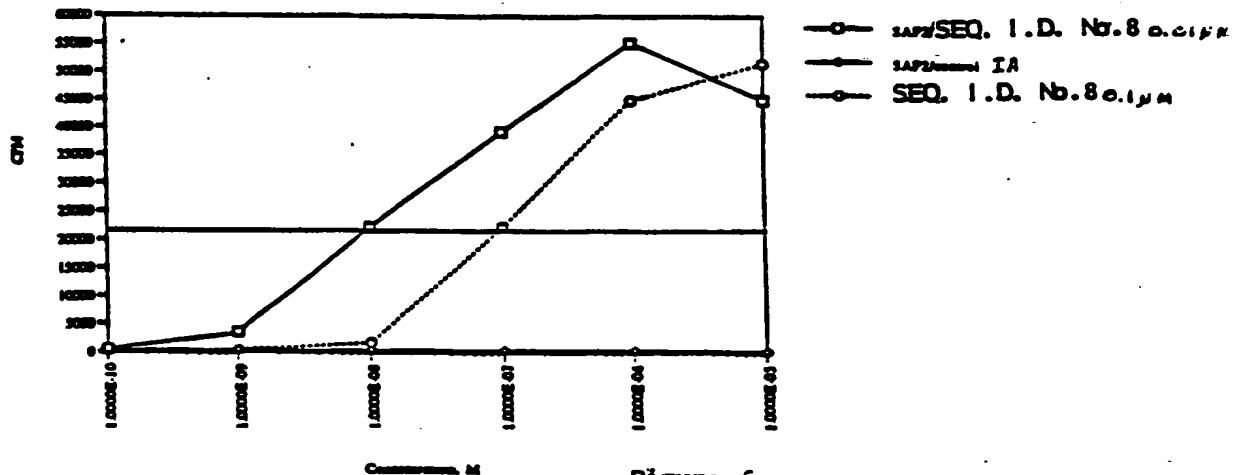


Figure 6

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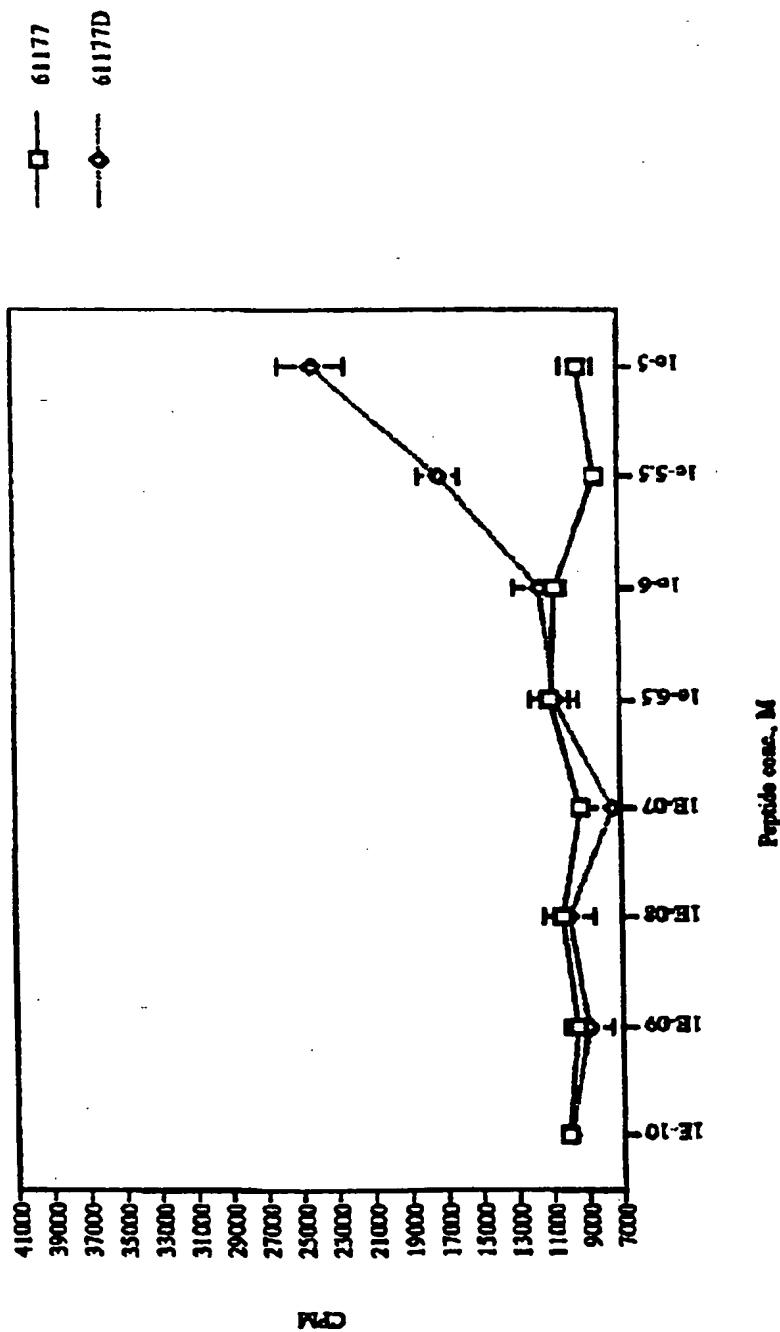


Figure 6
Panel D

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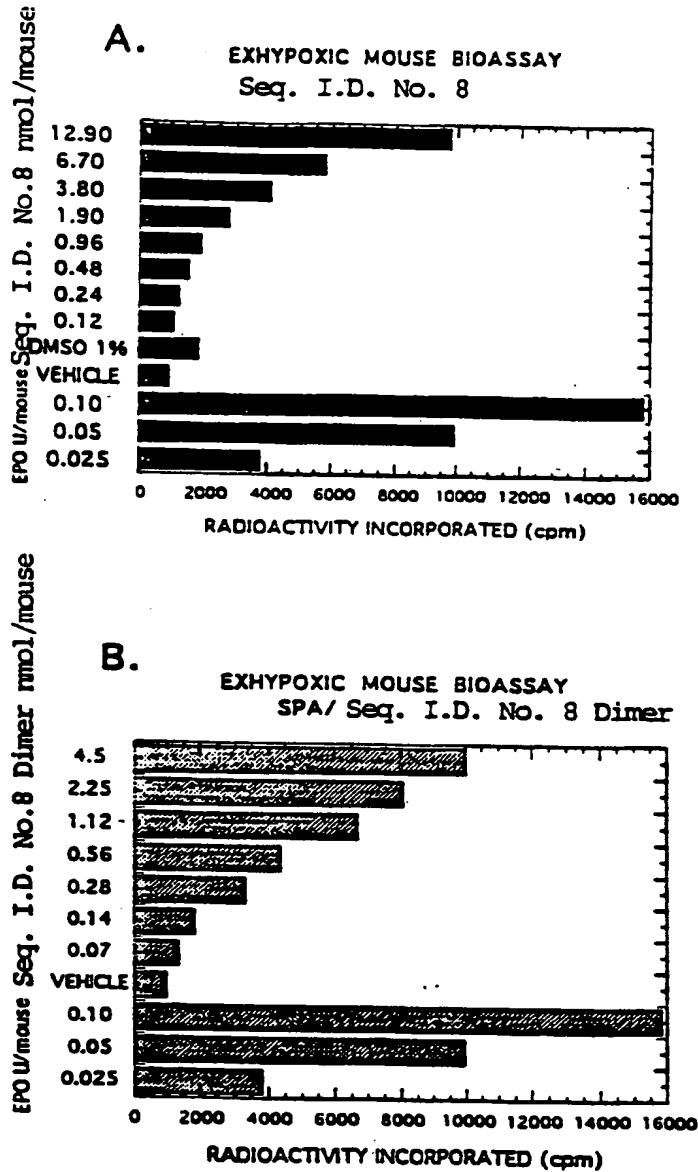


Figure 7

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CPM

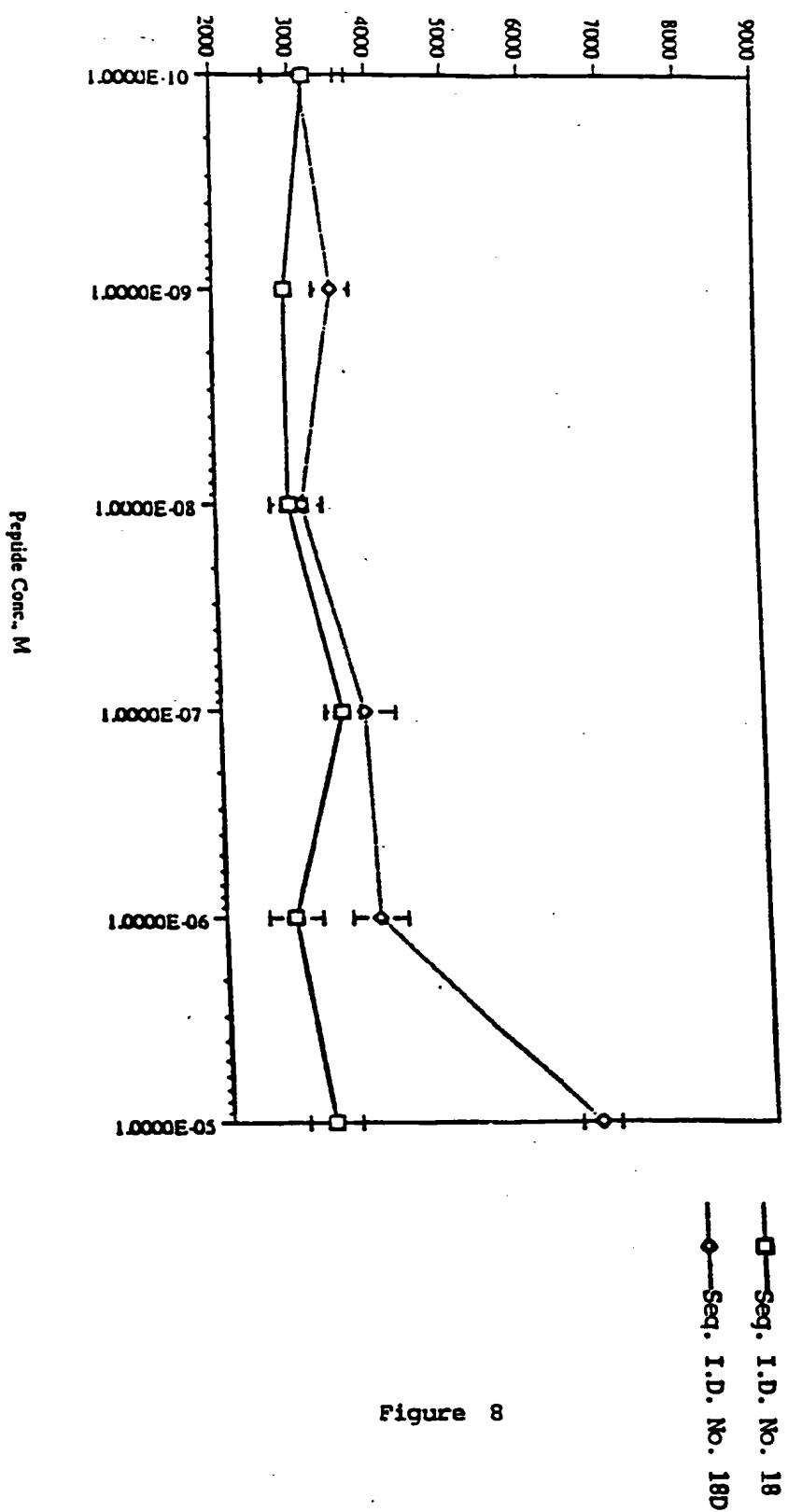


Figure 8

FIG. 9 (A)

Ac-GGTYSCHFGPLTWVCKPQGG	SEQ ID NO: 20
GGTYRCMGPMTWVCLPMGG	SEQ ID NO: 21
GGMYSCRMGPMTWVCGPSGG	SEQ ID NO: 22
GGWAWCRMGPITWVCSAHGG	SEQ ID NO: 23
GGMYSCRMGPNTWVCIPYGG	SEQ ID NO: 24
GGEYKCYMGPITWVCKPEGG	SEQ ID NO: 25
GGDYTCMGPMTWICTATGG	SEQ ID NO: 26
GGNYLCRFGPGTWDCTGFRG	SEQ ID NO: 27
GGNYVCRMGPITWICTPAGG	SEQ ID NO: 28
GGKDVCRMGPITWDCRSTGG	SEQ ID NO: 29
GGSYLCRMGPPTWLCTAQRGGGN	SEQ ID NO: 30
GGNYLCMGPATWVCGRMGG	SEQ ID NO: 31
GGEYKCRMGPLTWVCQYAGG	SEQ ID NO: 32
GGDYTCRMGPMTWICTATRG	SEQ ID NO: 33
GGVYVCRMGPWTWECTASGG	SEQ ID NO: 34
GGEYSCRMGPMTWVCSPTGG	SEQ ID NO: 35
GGEYLCRMGPITWVCERYGG	SEQ ID NO: 36
GGNYICRMGPMTWVCTAHGG	SEQ ID NO: 37
GGDYLCRMGPATWVCGRMGG	SEQ ID NO: 38
GGLYLCRFGPVTWDCGYKGG	SEQ ID NO: 39
GGLYSCRMGPITWVCTKAGG	SEQ ID NO: 40
GGGYHCRMGPMTWVCRPVGG	SEQ ID NO: 41
GGTYSCHFGPLTWVCKPQGG	SEQ ID NO: 42
GGIYKCLMGPPLTWVCTPDGG	SEQ ID NO: 43
GGLYSCLMGPITWLCKPKGG	SEQ ID NO: 44
GGDYHCRMGPLTWVCKPLGG	SEQ ID NO: 45
GGDYSCRMGPPTWVCTPPGG	SEQ ID NO: 46
GGDYWCRMGPSTWECNAHGG	SEQ ID NO: 47
GGKYLCFSFGPITWVCARYGG	SEQ ID NO: 48
GGLYKCRLGPITWVCSPLGG	SEQ ID NO: 49
GGSYTCRFGPETWVCRPNNG	SEQ ID NO: 50
GGSYSCRMGPITWVCKPGGG	SEQ ID NO: 51
GGSYTCRMGPITWVCLPAGG	SEQ ID NO: 52
GGLYECRMGPMTWVCRPGGG	SEQ ID NO: 53
GGDYTCRMGPITWICTKAGG	SEQ ID NO: 54
GGVYSCRMGPPTWECNRYVG	SEQ ID NO: 55
GGAYLCHMGPITWVCRPQGG	SEQ ID NO: 56
GGEYSCRMGPNTWVCKPVGG	SEQ ID NO: 57
GGLYLCRMGPVTWECQPRGG	SEQ ID NO: 58
GGLYTCRMGPITWVCLLPGG	SEQ ID NO: 59
GGLYTCRMGPVTWVCTGAGG	SEQ ID NO: 60

FIG. 9 (B)

GGVYKCRMGPLTWECRPTGG	SEQ ID NO: 61
GGDYNCRFGPLTWVCKPSGG	SEQ ID NO: 62
GGSYLCRFGPTTWLCSSAGG	SEQ ID NO: 63
GGSYLCRMGPTTWVCTRMGG	SEQ ID NO: 64
GGSYLCRFGPTTWLCTQRGG	SEQ ID NO: 65
GGWVTCRMGPITWVCGVHGG	SEQ ID NO: 66
GGQLLCGIGPITWVCRWVGG	SEQ ID NO: 67
GGKYSCFMGPTTWVCSPVGRGV	SEQ ID NO: 68
GGWVYCRIGPITWVCDTNGG	SEQ ID NO: 69
GGMYYCRMGPMTWVCKGAGG	SEQ ID NO: 70
GGTTQCWIGPITWVCRARGG	SEQ ID NO: 71
GGPYHCRMGPITWVCGPVGG	SEQ ID NO: 72
GGEYRCRMGPISWVCSQPQGG	SEQ ID NO: 73
GGNYTCRFGPLTWECTPQGGGA	SEQ ID NO: 74
GGSWDCRIGPITWVCKWSGG	SEQ ID NO: 75
VGNYMCHFGPITWVCRPGGG	SEQ ID NO: 76
GGLYLCRMGPQTWMQCQPGGG	SEQ ID NO: 77
GGDYVCRMGPMTWVCAPYGR	SEQ ID NO: 78
GGWYSCLMGPMTWVCKAHRG	SEQ ID NO: 79
GGKYYCWMGPMTWVCSPAGG	SEQ ID NO: 80
GGYVMCRIGPITWVCDIPGG	SEQ ID NO: 81
GSCLQCCIGPITWVCRHAGG	SEQ ID NO: 82
GGNYFCRMGPITWVCCPSVG	SEQ ID NO: 83
GGEYICRMGPLTWECKRTGG	SEQ ID NO: 84
GGLYACRMGPITWVCKYMA	SEQ ID NO: 85
GGQYLCTFGPITWLCRGAGG	SEQ ID NO: 86
GGVYACRMGPITWVCSPLGG	SEQ ID NO: 87
GGYTTCRMGPITWVCSAHGG	SEQ ID NO: 88
GGTYKCWMGPMTWVCRPVGG	SEQ ID NO: 89
GGNYYCRFGPITFECHPTGG	SEQ ID NO: 90
GGEYLCRMGPMTWVCTPVGG	SEQ ID NO: 91
GGLYTCRMGPITWVCLPAGG	SEQ ID NO: 92
GGLYTCRMGPITWVCLPAGG	SEQ ID NO: 93



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(74) Agents: CIAMPORCERO, Audley, A., Jr. et al.; Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, NJ 08933-0001 (US).			

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(54) Title: AGONIST PEPTIDE DIMERS

(57) Abstract

The present invention is directed to the dimerization of agonists and antagonists of cell surface receptors and particularly to peptide dimers which behave as cell surface receptor agonists in their dimeric form. Such receptors belong to the dimerization-mediated activation class often observed among receptors for growth and differentiation factors. The agonists of this class of receptors is understood to effect dimerization of the receptor and thus signal initiation. The present invention exemplifies dimers of erythropoietin (EPO) agonists and antagonists comprising a core amino acid sequence of $X_3X_4X_5GPX_6TX_7X_8$ (SEQ ID NO: 1) wherein each amino acid is indicated by standard one letter abbreviation; X_3 can be C, A, α -amino- γ -bromobutyric acid or Hoc; X_4 can be R, H, L or W; X_5 can be M, F, or I; X_6 is independently selected from any one of the 20 genetically coded L-amino acids or the stereoisomeric D-amino acids; X_7 can be D, E, I, L or V; and X_8 can be C, A, α -amino- γ -bromobutyric acid or Hoc, provided that either X_3 or X_8 is C or Hoc.

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INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/US 96/09469

A. CLASSIFICATION OF SUBJECT MATTER				
IPC 6	C07K7/06	C07K7/08	C07K14/00	C07K14/505
	C07K1/107	A61K38/04	A61K38/16	C07K14/52
				A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 23550 A (GENENTECH INC ;GODOWSKI PAUL J (US)) 25 November 1993 see page 1 - page 34; claims 1-52 ---	15-19, 26-28 25
X	WO 95 11987 A (INCYTE PHARMA INC ;SCOTT RANDY W (US); BRAXTON SCOTT M (US)) 4 May 1995 see page 51 - page 61; claims 30-37; examples F,H,I ---	15-18
Y	WO 90 08822 A (GENETICS INST ;WHITEHEAD INST (US)) 9 August 1990 see page 12, line 4 - page 12, line 27; claim 14 ---	25
X		26,28 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

5 March 1997

Date of mailing of the international search report

17.06.97

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GROENENDIJK, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/09469

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ACTA ENDOCRINOLOGICA, vol. 122, no. 2, February 1990, pages 241-248, XP000618626 P.BROSTEDT E.A.: "Characterization of dimeric forms of pituitary growth hormone by bioassay, radioreceptorassay and radioimmunoassay" see the whole document	15-17,19
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P,X	---	15-17, 19,25
P,X	WO 95 25746 A (NEW ENGLAND DEACONESS HOSPITAL) 28 September 1995 see the whole document	15-17, 19,25
A	---	1-30
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A	---	1-30
A	US 4 618 598 A (CONN P MICHAEL) 21 October 1986 see the whole document	1-30
A	---	1-30
A	G.JUNG E.A.: "peptides 1988; Proc. 20th Eur.Pept.Symp." 1989 , WALTER DE GRUYTER , BERLIN XP002021037 H.Kessler e.a.; Dimerization of cyclic hexapeptides; strong increase of biological activity see page 664 - page 666	1-30
A	---	1-30
A	WO 94 17099 A (CELTRIX PHARMA) 4 August 1994 The whole document; see especially Tables 1,2 and 4	1-30
A	---	1-30
4	V.J.HRUBY: "Peptide, structure and function; Proc.8th Am.Pept.Symp." 1983 XP002021038 R.J.Vavrek e.a.;Succinyl bis-bradykinins: potent agonists ... see page 381-384	1-30

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INTERNATIONAL SEARCH REPORT

Int	ional Application No
PCT/US 96/09469	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	<p>SCIENCE, vol. 273, 26 July 1996, LANCASTER, PA US, pages 464-471, XP002021035</p> <p>O.LIVNAH E.A.: "Functional mimicry of a protein hormone by a peptide agonist: the EPO receptor complex at 2.8 angström" see the whole document</p> <p>---</p>	1-30
T	<p>SCIENCE, vol. 273, 26 July 1996, LANCASTER, PA US, pages 458-463, XP002021036</p> <p>N.C.WRIGHTON E.A.: "Small peptides as potent mimetics of the protein hormone erythropoietin" see the whole document</p> <p>-----</p>	1-30

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/09469

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 8, 15-25
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 8 and 8-15 are directed to or encompass a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

subjects 1. + 2. (see continuation-sheet)

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/USA/210

Subject 1:claims 1-14,18,20-24,28-30(complete);15-17,25,26(all partially)

Compounds defined in the claims 1-6, their preparation and use and the methods defined in the claims 15-18,25,26 and 28, wherein the cell surface receptor is EPO-R.

Subject 2:claims 15-17,19,25-27(all partially)

The methods defined in the claims 15-19 and 25-28, wherein the cell surface receptor (ant)agonist is a GH (ant)agonist.

Subject 3:claims 15-17,19,25-27(all partially)

The methods defined in the claims 15-19 and 25-28, wherein the cell surface receptor (ant)agonist is a PDGF (ant)agonist.

Subject 4:claims 15-17,19,25-27(all partially)

The methods defined in the claims 15-19 and 25-28, wherein the cell surface receptor (ant)agonist is a EGF (ant)agonist.

Subject 5:claims 15-17,19,25-27(all partially)

The methods defined in the claims 15-19 and 25-28, wherein the cell surface receptor (ant)agonist is a G(M)-CSF (ant)agonist.

Subject 6:claims 15-17,19,25-27(all partially)

The methods defined in the claims 15-19 and 25-28, wherein the cell surface receptor (ant)agonist is a TPO (ant)agonist.

Subject 7:claims 15-17,19,25-27(all partially)

The methods defined in the claims 15-19 and 25-28, wherein the cell surface receptor (ant)agonist is a VEGF (ant)agonist.

Subject 8:claims 15-17,19,25-27(all partially)

The methods defined in the claims 15-19 and 25-28, wherein the cell surface receptor (ant)agonist is a FGF (ant)agonist.

Subject 9:claims 15-17,19,25-27(all partially)

The methods defined in the claims 15-19 and 25-28, wherein the cell surface receptor (ant)agonist is an insulin (ant)agonist.

Subject 10:claims 15-17,19,25-27(all partially)

The methods defined in the claims 15-19 and 25-28, wherein the cell surface receptor (ant)agonist is a IL-3 (ant)agonist.

FURTHER INFORMATION CONTINUED FROM PCT/USA/210

Subject 11:claims 15-17,19,25-27(all partially)

The methods defined in the claims 15-19 and 25-28, wherein the cell surface receptor (ant)agonist is a IL-5 (ant)agonist.

Subject 12:claims 15-17,19,25-27(all partially)

The methods defined in the claims 15-19 and 25-28, wherein the cell surface receptor (ant)agonist is a IL-6 (ant)agonist.

Subject 13:claims 15-17,19,25-27(all partially)

The methods defined in the claims 15-19 and 25-28, wherein the cell surface receptor (ant)agonist is a IL-2 (ant)agonist.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. J. of Patent Application No
PCT/US 96/09469

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